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Editorial

Traditional Systems of Medicine have received renewed global attention as these open new vistas of research for treatment of many diseases having no satisfactory cure in modern medicine, thus far. Unani system of medicine, although originated in Greece, is one of the recognized systems of medicine of the country. Although, the Unani medicine have been in use for centuries and are known for their therapeutic efficacies, there is a need to scientifically establish their efficacy and safety in order to achieve global acceptance. Organized research work in this system was, therefore, a need of the hour. In post independent era, Central Council for Research in Unani medicine, through its clinical, drug research, literary research, survey & cultivation of medicinal plants programme is contributing significantly for last three decades. *Vitiligo, Sinusitis, Filariasis, Eczema, Malaria, Infective Hepatitis, Asthma,* are some of the conditions where Unani therapies have earned recognition after scientific validation.

The Council has been publishing the peer reviewed *Hippocratic Journal of Unani Medicine* (HJUM), mainly to bring out fundamental and applied aspects of Unani Medicine. The journal also publishes recent advances in other related sciences and traditional medicines as well as different streams of medical sciences, which have bearing on validation and scientific interpretation of various concepts and strengths of Unani medicine.

In view of an overwhelming response, the journal earlier published twice a year, its periodicity has now been changed to quarterly w.e.f. January 2008 to accommodate more articles for quick dissemination of research data among scientific community. The journal has sufficient room for invited articles from luminaries of modern medicine and sciences as well as scholars of Unani medicine. The broad areas being covered include clinical research on single and compound Unani drugs, validation of regimental therapy, Clinical and experimental pharmacological studies, standardization of single and compound drugs, development of standard operating procedures, ethnobotanical studies, experimental studies on medicinal plants and development of agro-techniques thereof, and literary research on classics of Unani medicine. The journal is also open for studies on safety evaluation of Unani and other herbo-mineral drugs, nutraceuticals, cosmotherapeutics, aromatics, oral health, life style disorders, sports medicine etc. and such other newer areas which are the outcome of modern day living.

The current issue of this journal provides 14 original and review papers in the areas of *clinical research, drug standardization, pharmacology, ethnobotany* and allied disciplines contributed by eminent scholars in their respective fields. It is hoped that data presented will contribute significantly in R&D sector of traditional drugs and prove to be an excellent exposition of current research efforts of scientists in this direction. Council acknowledges the authors for their contributions included in this issue and hope for their continued support in this endeavor. We wish to ensure the readers to bring out the future issues of the journal on time.

We at the CCRUM have been constantly striving to reach to higher standards and make HJUM the leading journal of Unani medicine and related sciences. In this context, we thank our learned reviewers for their invaluable inputs in improving the manuscripts. We sincerely hope and trust that the mission can be accomplished with active partnership of quality-conscious individuals and institutions. Through these lines we seek your cooperation and support in materializing our dreams about the HJUM. In this regard, we request you for your as well as your colleagues' contributions for publication in and subscription to the journal. Further, we will appreciate if the journal is introduced far and wide. We would also welcome esteemed suggestions for achieving the highest standards of quality for the journal.

January 23, 2015



(Prof. S. Shakir Jamil)
Editor-in-Chief

The Effect of *Qurs-e-Zarishk Sagheer* (A Compound Unani Formulation) on Liver Enzymes in CCl_4 Induced Hepatotoxicity in Rats

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Abstract

Qurs-e-Zarishk Sagheer (QZS) is a pharmacopoeal compound preparation known to possess hepatoprotective effect. Present study was designed to evaluate its protective and curative potential on liver enzymes in CCl_4 (2 ml/kg of body weight i.p.) induced hepatotoxicity in rats. Ingredients of QZS in powdered form and its hydro alcoholic extract were used in the doses of 700 mg/kg, and 230 mg/kg body weight /day, respectively. Silymarin was used as standard drug in the dose of 100 mg/kg orally/day. Biochemical parameters including Serum Glutamate Oxaloacetate Transaminase, Serum Glutamate Pyruvate Transaminase and TBARS were determined along with the histological studies of liver tissues of all the animals. The elevation of marker enzymes and structural changes in histological reports of liver sections were taken as the indicators of hepatic injury. The study showed gross elevation of liver enzymes and histological changes in CCl_4 treated animals, while the test drug in both doses forms showed significant enzymes lowering activity, which was comparable with that of Silymarin. Biochemical parameters showed better results in respect of extract while hisptopathological observations were almost similar in both groups. The study demonstrated that QZS possesses significant liver enzyme lowering effect in CCl_4 induced hepatic injury indicating hepatoprotective effect.

Keywords: Pharmacopoeal compound, Hepatoprotective, CCl_4, Ethanolic extract

Introduction

Liver is the main organ to metabolize all the foreign compounds and has tremendous capacity to detoxify toxic chemicals of the body that is why it is susceptible to almost as many different diseases, and damage to the liver inflicted by hepatotoxic agents is of grave consequences (Subramanian *et al.,* 1999). In today's world, liver is overwhelmed with toxin problems, alcohol abuse and susceptibility to viral infections, immune disorders or problems of metabolism, leading to serious liver disorders such as cirrhosis, jaundice, tumors, metabolic and degenerative lesions, liver cell necrosis and hepatitis etc. amongst them cirrhosis, fatty liver and hepatitis are important in world health issues (Luper, 1998). The magnitude of derangement of the liver by diseases or hepatotoxins and the extent of hepatic damage are generally assessed by measuring the activity of liver enzymes namely SGOT, SGPT and TBARS (Morrison *et al.*, 1983). The decreased levels of transaminases indicate stabilization of plasma membrane and protection of hepatocytes against damage caused by hepatotoxin. The modern synthetic drugs have very little to offer for alleviation of hepatic ailments and some

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of these drugs even adversely affect the liver function (Meyer *et al.*, 2001 and Harsh Mohan, 2002). In recent years, the use of alternative drugs for the treatment of liver diseases has increased all over the world. The natural drugs are believed to be harmless and free from serious adverse reactions and also the limited therapeutic options and disappointing therapeutic success of modern medicine has increased the usage of alternative medicine including their preparations (Stickel *et al.*, 2007). The Unani system of medicine on account of having proven ability of bringing the marker enzymesas to the normal level has a major role in the treatment of liver ailments. It possesses a number of single and compound drugs that are considered highly effective and safe. Few of these drugs have been investigated and shown to possess significant hepatoprotective effect (Ghufran *et al.*, 2002; Anusha *et al.*, 2011; Zafar and Ali., 1998: Akhtar *et al.*, 2009; Handa and Sharma, 2002). *Qurs-e-Zarishk Sagheer* (QZS) is one such compound preparation described to be effective in liver diseases (Khan, 1921) and prescribed commonly by the physicians of Unani medicine. But it has not been investigated for its effect in hepatic diseases specially its effect on marker enzymes of liver function. Therefore the present study was undertaken to evaluate the efficacy of test drug on various liver enzymes in rats subjected to acute hepatotoxicity by the administration of Carbon tetrachloride. The study was designed in such a way that both curative and protective effects can be evaluated.

Materials and Methods

Ingredients of QZS (Khan, 1921)

1.	Zarishk	(*Berberis aristata*)	44.5 gm
2.	Behdana	(*Pyrus cydonia*)	44.5 gm
3.	Tukhm-e- Kasni	(*Cichorium intybus*)	10.5 gm
4.	Tukhme Khurfah	(*Portulaca oleracea*)	10.5 gm
5.	Tukhme Kheera	(*Cucumis sativus*)	10.5 gm
6.	Tukhme Kakdi	(*Cucumis melo*)	10.5 gm
7.	Gule surkh	(*Rosa demescena*)	17.5 gm
8.	Rewande cheeni	(*Rheum emodi*)	3.5 gm
9.	Balchad	(*Nardostachys jatamansi*)	3.5 gm

Preparation and Dosing of Test Drug

The ingredients of QZS were purchased from the herbal market of Aligarh and New Delhi. Pharmacognosy section of Department of Ilmul Advia, Aligarh Muslim University, Aligarh authenticated the samples. All the crude drugs of QZS were pulverized to get a fine powder which was homogenized in water before administration to the animals. A 50% ethanol extract was also prepared through Soxhlets Apparatus (Anonymous, 1968; Anonymous, 1987). The extract was dried

over a hot plate until a semi solid preparation was collected. The dried extract was however reconstituted in distilled water before the administration and a homogenous suspension was given to the animals orally with the help of gastric canula. The dose for albino rats was determined after multiplying the human dose by the conversion factor of 7 (Dhawan, 1982). The dose of QZS thus, calculated for an albino rat was found to be 700 mg/kg and 230 mg/kg of powder drug and the extract, respectively.

Chemicals

CCl_4, n-butanol, Acetic acid were purchased from Thomas Baker Pvt. Ltd. Mumbai, Sodium dodecyle sulphate and Thiobarbituric from Otto Kemi Mumbai, 1, 1, 3, 3-tetraethoxypropane from Sigma USA, Silymarin from Sigma-Aldrich, Germany, Folin's reagent from CDH, Mumbai, AST and ALT estimation kits from Span Diagnostic Ltd, Surat; Olive oil and Formalin were purchased from SD Fine chemicals, Chennai. All the reagents used were of analytical grade.

Animals

Albino rats of either sex with weight range of 125-175 gm were used for experiment. The rats were randomly selected and were divided into five groups of 6 animals each. So, total 60 animals were utilized in the two test meant for Protective and Curative study. They were housed in clean polypropylene cages and the room temperature was maintained at 25 ± 2°C with 12 hour light and dark cycle. All the animals received standard diet (Amruta Labs, Pune) and water *ad libitum*. The animals were deprived of food for 12 hours before the treatment. The experimental protocol was approved by the Institutional Ethics Committee.

Experimental Design

The animals were divided into 5 groups of six animals each for the protective and curative test and were treated as follows:

Groups	Treatment
Group I (Plain Control)	Vehicle daily for 7 days
Group II (Negative control)	CCl_4 (0.2 ml/100 gm)
Group III (Standard control)	Silymarin (10 mg/100 gm) for 7 days+ CCl_4 (0.2 ml/100 gm)
Group IV (Test groups) (powder)	QZS Powder (70 mg/100 gm) for 7 days+ CCl_4 (0.2 ml/100 gm)
Group V (Test group) (extract)	QZS Extract (23 mg/100 gm) for 7 days+ CCl_4 (0.2 ml/100 gm)

The animals were treated with CCl_4 on second day in the test for preventive effect while in the test designed for curative effect CCl_4 was given on day 6. Other treatments were similar in both the tests. On the 8^{th} day all the rats were sacrificed under ether anesthesia and blood was collected from each animal for serum analysis and liver were removed and fixed in 10% formalin for histopathological studies of the liver to determine the degree of hepatic damage (Devaraj *et al.*, 2011).

Preparations of Samples for Biochemical Studies

The blood and liver were collected after sacrificing the animals. The blood was kept for 30 minutes without disturbing and was then centrifuged for 15-20 minutes at 5000 rpm to separate the sera. It was stored at 4^0C and ALT, AST (Reitman and Frankel 1957), and TBARS (Okhawa *et al,* 1979) which is an index of lipid peroxides (Lowry *et al.,* 1951) were determined.

Histopathological Observation

The liver of rats was removed immediately after sacrificing them and fixed in 10% formalin. Care was taken to keep the volume of the fixative (Mukherjee, 1988). The tissue was processed and sections were cut. The slides were prepared and stained with haematoxyline and eosin stain and the histopathological features were observed by a photomicroscope under various magnifications.

Statistical Analysis

Data was presented as mean ± Standard Error and analyzed using one way ANOVA test, followed by pair-wise comparison of various groups by LSD. The analysis was carried out by using the software of the website 'analyseit.com'.

Results

CCl_4 in dose of 2 ml/kg produced acute hepatic damage in negative control group when compared with normal control. There was significant rise in the level of enzymes SGOT, SGPT, and also the Thiobarbituric acid reactive substances (TBARS) as compared to plain control. The concentration of Malondialdehyde (MDA), SGOT and SGPT in CCl_4 treated animals in protective group was found to be 4.92±0.45 (ç mole of MDA / mg of protein), 111.7 ± 3.60 (u/ml) and 97 ± 6.61 (u/ml), whereas, the concentration in plain control of protective group was found to be 1.18± 0.095 (ç mole), 26.3 ± 2.94 (u/ml) and 27.7± 3.40 (U/ml), respectively that was significantly less than that in the CCl_4 group (P<0.001). The standard Silymarin showed significant reduction in all parameters when compared with CCl_4 treated group. Treatment with two samples of test drug along with CCl_4

intoxication showed decrease in level of marker enzymes. The values for SGOT, SGPT were found to be within normal limit. A similar pattern was also followed in curative group of animals (P<0.001). The animals treated with two samples of test drug (group IV & V) did not show any significant rise in MDA, SGOT & SGPT (Table-A & B and Fig 1&2).

Table A: Protective effect of QZS in CCl_4 mediated hepatic damage

	Groups	**TBARS (η mole of MDA / mg Protein)**	**SGOT (Units/ml)**	**SGPT (Units/ml)**
Group I	Plain Control	1.18 ± 0.095	26.3 ± 2.94	27.7 ± 3.40
Group II	CCl_4 (0.2ml/ 100gm)	4.92 ± 0.45	111.7 ± 3.60	97 ± 6.61
Group III	Silymarin (10mg/100gm)	1.48 ± 0.05	40.3 ± 3.40	44.5 ± 2.06
Group IV	TD *(QZ)* Crude (70mg/100gm)	2.61 ± 0.19 a^3	41 ± 3.39 a^3	44.3 ± 2.08 a^3
Group V	TD *(QZ)* Extract (23mg/100gm)	2.43 ± 0.15 a^3	34.7 ± 3.36 a^3	18.3 ± 1.69 a^3 c^3

(n=6)

1 = P < 0.05, 2 = P<0.01, 3 = P<0.001

a= against CCl_4, b=against plain control, c=against Silymarin

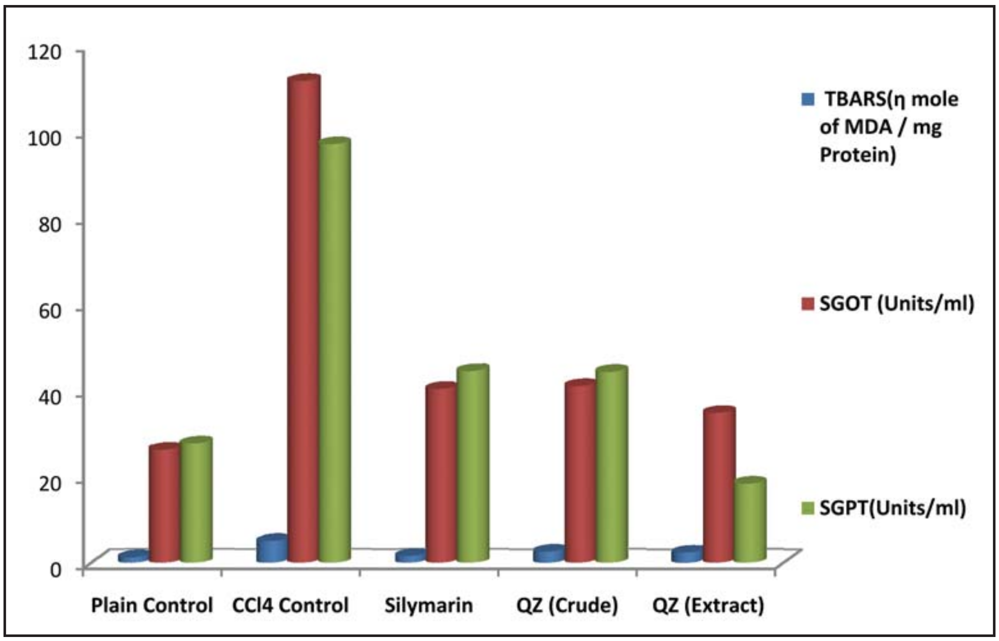


Figure 1

Table B: Curative effect of QZS in CCl_4 mediated hepatic damage

Groups	TBARS (η mole of MDA / mg Protein)	SGOT (Units/ml)	SGPT (Units/ml)
Plain Control	1.96 ± 0.13	30.7 ± 2.40	24.7 ± 2.58
CCl_4 (0.2ml/100gm)	5.46 ± 0.45	114.8 ± 3.43	100.2 ± 4.05
Silymarin (10mg/100gm)	1.68 ± 0.10	46.2 ± 2.10	41.8 ± 2.74
TD *(QZ)* Crude (70mg/100gm)	2.07 ± 0.15 a^3	60.3 ± 3.84 a^3	54.3 ± 2.19 a^3
TD *(QZ)* Extract (23mg/100gm)	2.52 ± 0.20 a^3	46.5 ± 4.24 a^3	48.7 ± 3.84 a^3

(n=6)

1 = $P < 0.05$, 2 = $P<0.01$, 3 = $P<0.001$

a= against CCl_4, b=against plain control, c=against Silymarin

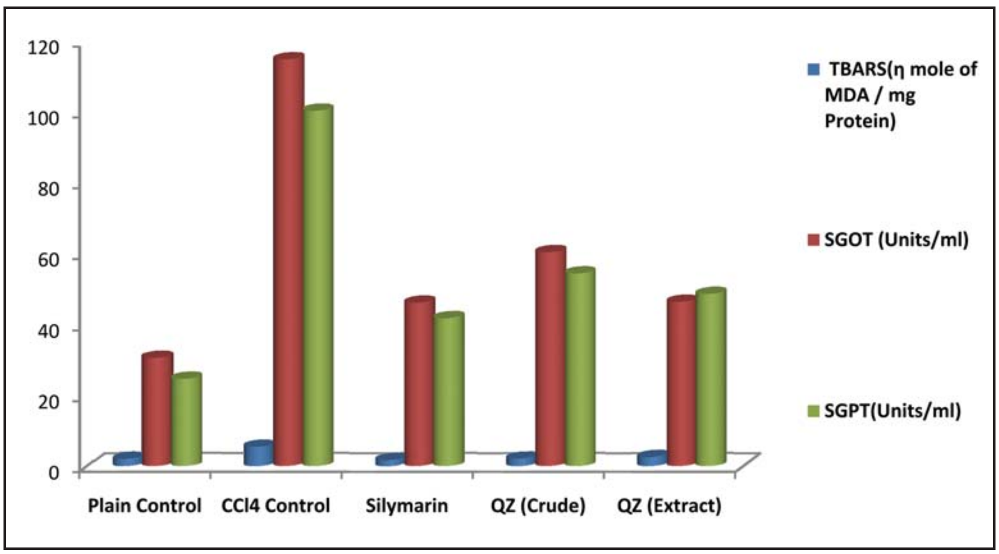


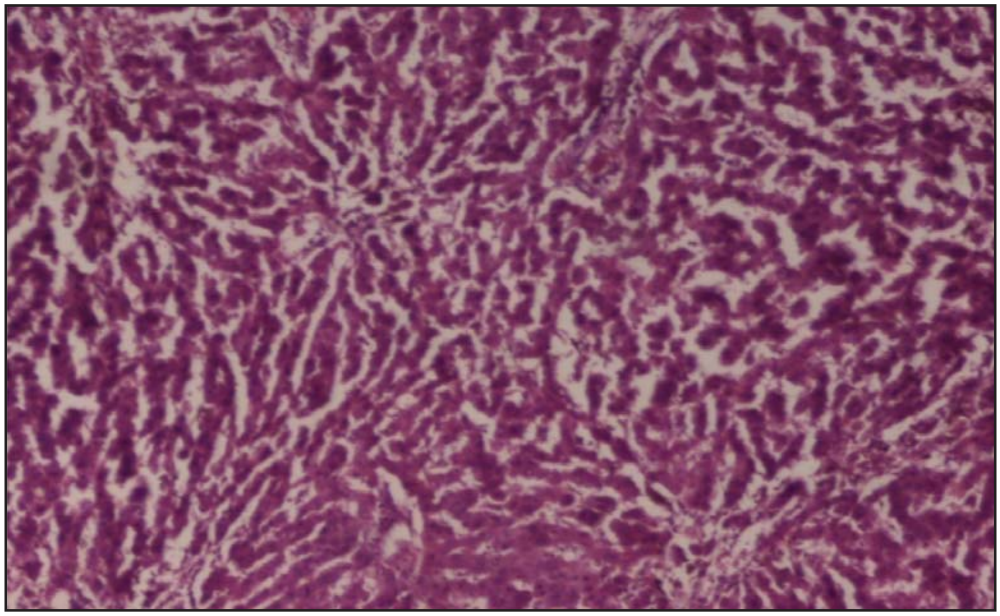
Figure 2

Histopathology

The histopathological studies of the liver showed centrilobular necrosis and vascular congestion with mononuclear cell infiltration in CCl_4 control rats. CCl_4 treatment caused marked congestion of central vein and portal triads, indicating fibrosis (Fig 4), in comparison with normal control where, central blood vessels and radiating cords of hepatocytes as well as the vascular sinusoids were observed

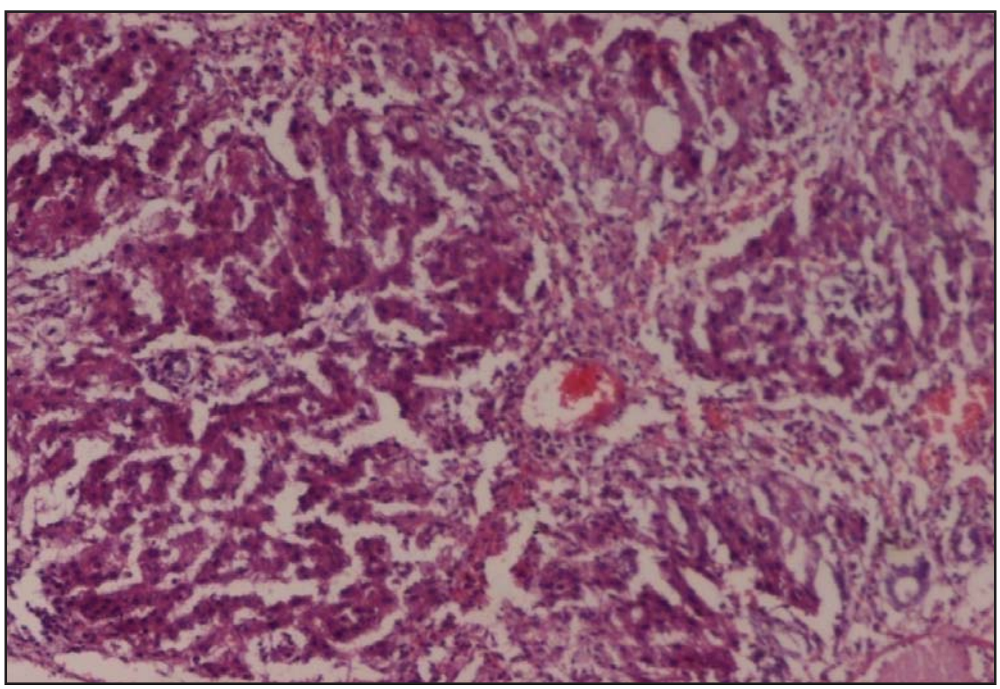
with no evidence of fatty changes, necrosis or inflammation (Fig. 3). The animals treated with Silymarin showed almost normalization of fatty accumulation and necrosis (Figure 5). Animals treated with crude powder exhibited intact

Figure 3: Plain control (Water only)



Central blood vessels and radiating cords of hepatocytes as well as the vascular sinusoids with no evidence of fatty changes, necrosis or inflammation.

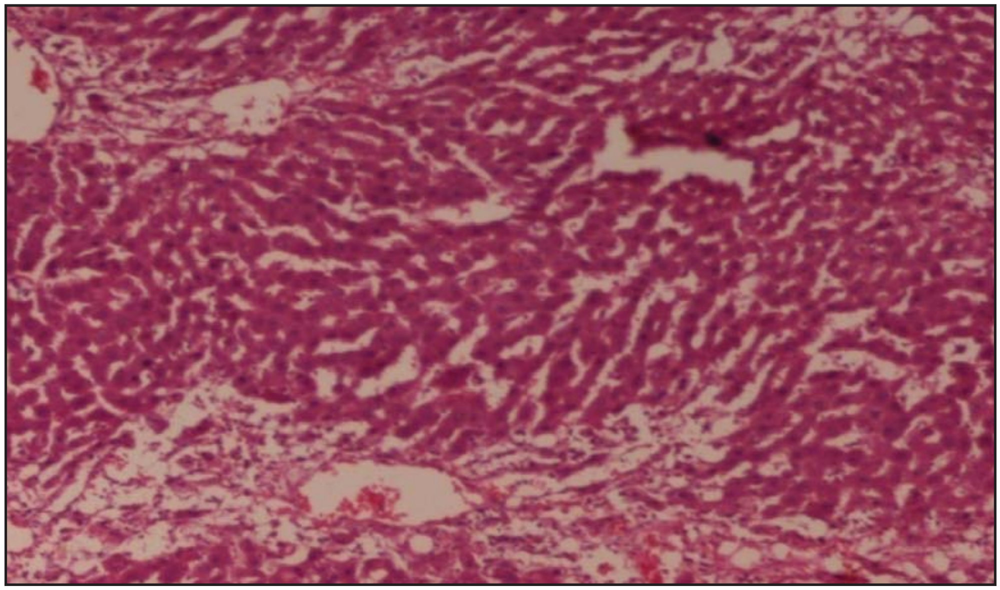
Figure 4: Negative Control (CCl_4 only)



Centrilobular (Acidophilic) necrosis and vascular congestion

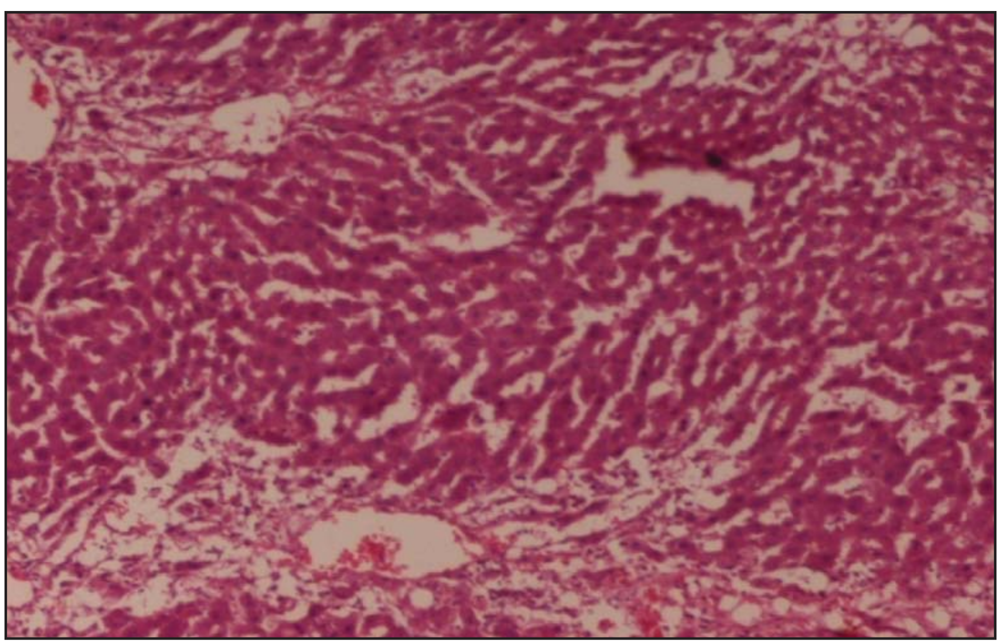
hepatocytes, some congestion in portal triad. The group received extracts form of test drug showed minimal degree of edema, normalization of fatty changes as well as normalization of necrosis of the liver. A substantial protection against hepatic damage was achieved by the two samples of test drug (Fig. 6 & 7). Both the

Figure 5: Standard (Silymarin) + CCl4



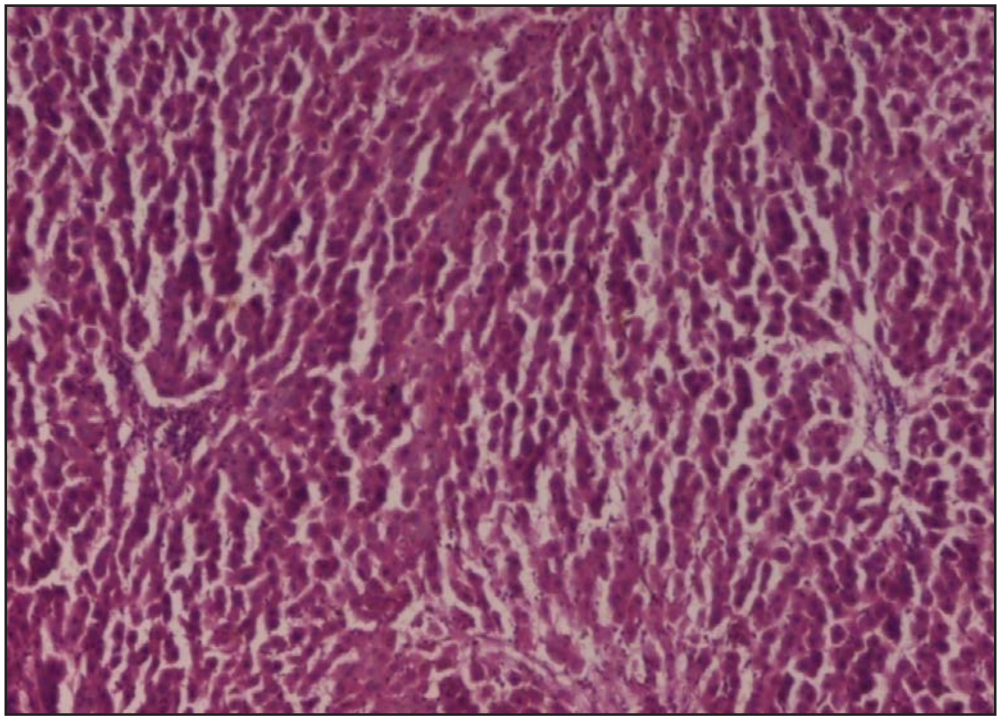
Mild vascular congestion and peri-vascular infiltrate of mono nuclear cells and fibroblast. No fatty changes

Figure 6: QZS (powder) + CCl_4 treated



Intact hepatocytes, congestion in Portal Triad, edema minimal

Figure 7: QZS (Extract) + CCl_4 treated



Edema+, no inflammatory cell no cholestasis, Kupffer cells present

dosage forms prevented CCl_4-induced changes in liver. The Silymarin and test drug treated groups showed excellent protection and cure to liver architecture.

Discussion

The present study was under taken to determine the concentration of serum marker enzymes of liver and that of TBARS against hepatic injury produced by carbon tetrachloride in rats. Administration of CCl_4 increased the concentration of SGOT, SGPT and TBARS significantly as compared to their normal values. The enzymes leaking out from damaged liver cells into circulating blood represent the damage to hepatic cells. The extent of hepatic damage was assessed by the elevation in the release of cytosolic transaminases (SGPT and SGOT) in circulation, as the CCl_4 administration is reported to cause marked elevation in serum enzymes. A high level of SGOT indicating cellular damage is frequently observed in cases of viral hepatitis, myocardial infarction and muscle injury etc (Muriel *et al.*, 1992). The release and thereby the high concentration of SGPT in the blood followed the similar pattern as that of SGOT. However since it is mostly present in hepatocytes therefore, SGPT is considered more reliable and comparative better parameter to detect the liver injury (Agarwal *et al.*, 2006). Carbon tetrachloride (CCl_4) mediates changes in liver function that ultimately leads to destruction of hepatocellular membrane. Cytochrome P-450 activates CCl_4 to form various free

radicals (trichloromethyl, Cl_3 C-CCl_3 (hexachloroethane), $COCl_2$ (phosgene), which are involved in pathogenesis of liver damage in chain reactions causing peroxidation of lipids, covalent binding to macromolecules, disruption of metabolic mechanisms in mitochondria, decrease in phospholipids, increase in triglyceride, inhibition of calcium pump of microsomes thus leading to liver necrosis (Maryam *et al*., 2011) and subsequently liver fibrosis and cirrhosis (Waer *et al.*, 2012). Since, the pathological lesions develop in CCl_4 treated animals closely resemble the symptoms of acute viral hepatitis and cirrhosis in human therefore it serves as an excellent model to assess the efficacy of any drug having hepatoprotective potential (Desai, 2011). QZS has shown very significant reduction in the concentration of enzymes in both the models which is comparable with the results of standard drug (Sylimarin). The extract form has demonstrated slightly better results as compared to powder form in respect of biochemical parameters but no significant difference was observed in histological findings. In the histological study it was observed that QZS caused greater retention of hepatic architecture, reduction in fatty degeneration and necrosis in comparison of CCl_4 treated animals. The two dosage forms of QZS were able to reduce the level of enzymes especially SGOT in both the experiments, indicating that they protected the hepatocytes and maintained the normal liver physiology and the functional status of the liver, and further caused stabilization of plasma membrane and even regeneration of damaged liver cells. Since the test drug has shown both protective and curative effect as evidenced from the biochemical and histological findings therefore it can be proposed to be beneficial in different forms of hepatitis. The likely mechanism of hepatoprotective and hepatocurative effect appears to be antioxidant activity as both the dosage forms demonstrated significant antioxidant effect (TBARS).

Conclusion

The study demonstrated a significant protective and curative effect produced by QZS (both the extract and crude powder forms) in CCl_4 induced hepatic damage as it significantly decreased the level of marker enzymes of liver function, decreased the level of Thiobarbituric acid reactive substances and protected the liver tissue from any major damage. It also demonstrated a significant antioxidant effect possessed by the test drug; antioxidant effect may be one of the mechanisms of its hepatoprotective effect.

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Effect of a Combination of *Piper cubeba* L.f and *Cyperus rotundus* L. in Experimentally Induced Urolithiasis in Rats

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Abstract

The present study was undertaken to investigate the effect of a combination of Kabab Chini *(Piper cubeba*) and Sad Kufi *(Cyperus rotundus*) in urolithiatic rats. Thirty male Sprague Dawley rats divided into 5 groups of 6 animals each were used. Group I served as control and received regular rat food and drinking water *ad libitum*. The animals of group II to V were treated with Ethylene glycol (EG) 0.75% (V/V) and ammonium chloride (AC) 1% (W/V) by adding in their drinking water for 7 days to induce urolithiasis. The animals of group II were sacrificed just after 7 days of administration of EG and AC and served as Positive control A, while group III was left untreated till 14 days, and served as Positive control B. The animals of group IV were treated with Cystone (750 mg/kg) and served as Standard control, while the animals of group V were treated with hydro-alcoholic extract of the combination of *Piper cubeba* and *Cyperus rotundus* (135mg/kg) and served as Test group. The treatment continued further for next14 days. At the end of treatment all the animals were sacrificed after 24 hrs of fasting. Highly significant calcium oxalate stones were found in renal tissue as well as in the urine along with increase in urine and serum Calcium, Chloride, Sodium, Phosphorus and creatinine. Test drug significantly decreased the quantity of calcium oxalate deposited in the kidneys and also reverted all the biochemical changes induced by calcium oxalate. The effect of the test drug was found to be comparable to the standard drug Cystone. The study demonstrated a significant lithotriptic effect produced by the combination of *Piper cubeba* and *Cyperus rotunda*.

Keywords: *Kabab Chini, Sad Kufi,* Calcium oxalate, Ethylene glycol, Ammonium chloride, Lithotriptic.

Introduction

Urolithiasis is a common disease worldwide leaving no age, cultural, geographical or racial group spared (Moe, 2006). Calcium oxalate stones are most common occurring in more than 80% of urolithiatic patients. They are found either alone or mixed with calcium phosphate (Tiselius, 2003). A number of medicinal plants are used as medicine for the treatment of urolithiasis in Unani system of medicine. Badiyan *(Foeniculum vulgare),* Barge Turb (leaves of *Raphanus sativus),* Duqoo *(Pencedanum grande),* Habbul qilt *(Dolichos biflorus),* Khar khask *(Tribulus terrestris),* Darchini *(Cinnamomum zeylanicum*), Muqil *(Commiphora mukul),* Parsiaoshan *(Adiantum capillus veneris)* etc. are some of the drugs used commonly as single drugs (Razi 2001; Ali, 2004; Qamri, 2008), while some of the

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formulations possessing antilithiatic activity include *Sharbate Aloo Baloo, Sharbate Buzoori, Kushta Hajrul Yahood, Majoon Aqrab* etc (Anonymous, 2007; Hussain, 1844). Among them only few have been evaluated on scientific parameters and shown to possess antilithiatic activity (Hadjzadeh *et al*., 2007; Ahmed *et al.,* 2013; Shah *et al.,* 2011; Hussain, 2010; Shah *et al.,* 2012).

Kabab Chini (KC) and *Sad Kufi* (SK) are two important drugs used commonly in Unani System of Medicine for antilithiatic activity. Both the drugs possess *mufattite hisat* (lithotriptic), *muhallil* (resolvent), *mufattehe sudad* (deobstruent) *and mudirre baul* (diuretic) properties (Ghani, YNM; Chughtai and Chughtai, YNM; Baitar, 2002), and have been investigated for various pharmacological activities. In a recently concluded study KC and SK have been shown to possess dose dependent antilithiatic activity (Humairah *et al.*, 2014). In view of the attributed lithotriptic effect of these two drugs in Unani literature, and the findings of the recent study, the present study was undertaken to find out the synergistic effect, if any, between the two drugs in combination form in experimentally induced urolithiasis in male Sprague Dawley rats.

Materials and Methods

Animals

Male Sprague Dawley (SD) rats weighing 200-250 gm were procured from registered breeders. Prior to experiment the animals were allowed to get acclimatized for one week. They were maintained under standard laboratory conditions and provided standard diet and water *ad libitum* (unless stated otherwise). They were housed in clean polypropylene cages in a room, maintained at a temperature of 25±2^{0}C and humidity of 45-55% with 12 hour light and 12 hour dark cycle. The study was undertaken in the department of Ilmul Advia, National Institute of Unani Medicine (NIUM), Bangalore after getting the approval of Institutional Animal Ethics Committee (IAEC) of the institute vide registration no IAEC/IX/05/IA.

Chemicals and Reagents

Cystone ® manufactured by the Himalaya Drug Company was procured from the market. Ethylene glycol and gum acacia of analytical grade were purchased from NICE chemicals Pvt. Ltd., Kerala and Ammonium chloride from CDH Pvt. Ltd., New Delhi. Reagents used for Biochemical analysis, kidney homogenate preparation and for urinary parameters were Calcium (Arsenazo III method), Sodium (mono test) and Chloride Reagent set (mercuric Theocynaate method). These items were procured from Lab-care Diagnostics (India) Pvt. Ltd. Gujarat. Phosphorus (UV Molybdate method) was obtained from Pathozyme Diagnostic,

EURO diagnostic systems Pvt. Ltd. Chennai, India. Magnesium kit (Calmagite method) from Coral Clinical Systems, Uttarakhand, India and Urea kit (Urea kin-GLDH) was procured from Prism Diagnostics Pvt. Ltd. Thane, India. Creatinine (Modified Jaffe's method) was procured from AD, India, EURO diagnostic systems Pvt. Ltd.

Plant Material and Preparation of Extract

The fruit of KC was procured from the pharmacy of NIUM, Bangalore. The fresh rhizomes of SK were collected from Herbal garden of NIUM, Bangalore. The drugs were identified by a renowned Botanist. The voucher specimen was deposited in the herbarium of NIUM, Bangalore with reference number 15/IA/Res/2014.

The fruits of KC were kept in drying chamber at 40^{0}C for about 30 min to dry the moisture if any, and powdered coarsely in an electrical grinder. The rhizomes of SK were carefully washed and rinsed with tap water for at least 30 min. Dead parts were removed. Roots were separated from the rhizomes, and shade dried at room temperature of 28°C ± 1°C for 15 days. Dried rhizomes were pulverized in electric grinder in the form of coarse powder. The powder of both the drugs was extracted in Soxhlet's extractor separately with hydroalcoholic solvent (distilled water and ethanol 1: 1) for about 6 hours at a fixed temperature of 80^{0}C. The liquid extract was cooled and filtered by Whatman filter paper 40, the filtrate was then concentrated over steam bath (80^{0}C) till it dried. The resulting dark colour residue was collected and stored in refrigerator for further use.

Dosage of the Drug

The human therapeutic dose of KC and SK is 3 gm and 7 gm, respectively (Kabeeruddin 2007). The dose of the crude drug for Sprague Dawley rat was calculated by factor seven (Freirich et al., 1966) and found to be 350 mg/kg for KC and 816 mg/kg for SK. Since the hydroalcoholic extract of the test drugs was used so the dose of extract was calculated with respect to its yield percentage (10.06% for KC and 12.79% for SK). Thus the dose of extract was found to be 35 mg/kg for KC and 104.8 mg/kg for SK which was rounded off to 100 mg/kg. In order to know synergistic effect the calculated dose of the combination both the doses was taken in the study i.e. 135 mg/kg. The extract of test drug was reconstituted a fresh in water with 5% gum acacia, daily before the administration.

Ethylene Glycol and Ammonium Chloride Induced Urolithiasis

The experiment was carried out by the method of Fan *et al.*, 1999 and Aziz *et al.*, 2012 with some modification in the treatment schedule. Thirty male Sprague

Dawley rats divided into 5 groups of 6 animals each were used. Group I served as Plain control and received regular rat food and drinking water *ad libitum*. While the animals of group II to V were treated with Ethylene glycol 0.75% (V/V) and ammonium chloride 1% (W/V) added to their drinking water for 7 days to induce urolithiasis. All the animals received regular rat food. The animals of group II were sacrificed after 7 days of administration of ethylene glycol and ammonium chloride and served as Positive control A. While the animals in group III were left untreated after seven days administration of EG and AC till 14 days and served as Positive control B, thereafter they were sacrificed. From 8th day the animals of group I received 1 ml of 5% gum acacia, while the animals of group IV were treated with Cystone in the dose of 750 mg/kg, and served as Standard control. The animals of group V were treated with hydroalcoholic extract of the test drug and served as Test group. The test and standard drugs were suspended in 5% gum acacia. The treatment continued further for next 14 days. At the end of treatment all the animals were kept on fasting for 24 hrs and sacrificed thereafter.

Urine Analysis

Urine analysis was done on 8th day after administration of EG and AC and on 22nd day after treatment with test drug. The animals of each group were placed singly in individual metabolic cage with water *ad libitum* for the collection of urine passed during 24 hours. The calcium oxalate crystals present in urine were identified and counted using the light microscope (40X) (Kaur *et al*., 2009). One ml of urine sample was centrifuged at 2500 rpm for 5 min (Dhaval and Arvind, 2013) and analyzed for presence of calcium, phosphorus, creatinine, sodium, chloride and magnesium by autoanalyser.

Serum Analysis

On 22nd day rats were anaesthetized with Theopentone sodium (50 mg/kg IP). The abdomen was cut open and the blood samples were collected by cardiac puncture. Serum was separated by centrifugation at 10,000 rpm for 10 min (Dhaval and Arvind, 2013) which was analyzed for serum calcium, creatinine, urea and phosphorus.

Kidney Homogenate Analysis

After collection of blood both the kidneys were dissected out. Right kidneys from two animals of each group were sent for histopathological examination. While the left kidneys of all the rats were used for kidney homogenate analysis. Isolated left kidney was trimmed off from extraneous tissue then dried at 80^{0}C in a hot air oven for 30 min. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1 N hydrochloric acid for 30 min and homogenized using a tissue homogeniser.

The homogenate was centrifuged at 2,000 x g for 10 min (Dhaval and Arvind, 2013) and 6 ml of supernatant was discarded. Remaining supernatant was used for the analysis of calcium and phosphorus content.

Statistical Analysis

The data was analyzed using Graph pad software. ANOVA repeated measure with Tukey-Kramer multiple pair comparison test was applied for the parameters which passed normality test.

Results

Urine Analysis

In all groups after 7 days administration of EG and AC a significant increase ($p<0.001$) in number of calcium oxalate crystals was found when compared with plain control. On intra group comparison of the findings of 22nd day from 8th day, significant reduction ($p<0.001$) was noted in all the groups. The same level of reduction ($p<0.001$) was observed in the test groups on 22nd day when compared with positive control B. The number of crystals in test group after 14 days treatment with test drug was found almost equal to plain control on 8th day and 22nd day (Table 1, Fig. 1-6). In EG and AC treated groups urine calcium and phosphorus increased while creatinine and magnesium level decreased significantly. On 22nd day after treatment with test drug Ca decreased significantly ($p<0.05$). Urine Phosphorus also decreased significantly but magnesium level increased significantly ($p<0.001$) (Table 2).

Table 1: Effect of the test drug on calcium oxalate crystals in urine in "Ethylene glycol-Ammonium chloride induced Urolithiasis" in Rats

Groups	Drug & dose	No. of CaOx crystals	
		8th day	22nd day
Plain control	1 ml of 5% GA	7.5 ± 0.88	6.66 ± 0.66
Positive control A	EG 0.75% and AC1%	131.7 ± 10.93^{a***}	–
Positive control B	EG 0.75% and AC1%	130.8 ± 10.83 ^{a***}	92.50 ± 8.24 ^{a***}
Standard control	Cystone 750 mg/kg	132.7 ± 16.98 ^{a***}	8.33 ± 1.87 ^{b***c***}
Test group	combination of KC & SK 135 mg/kg	124.2 ± 18.09 ^{a***}	6.00 ± 0.68 $^{b*** c***}$

***$p<0.001$ **a**- compared with plain control, **b**-compared with Positive control A, **c**- compared with Positive control B. n=6

CaOx crystals seen in urine under light microscopy (40X)



Fig. 1: Plain control on 8th day

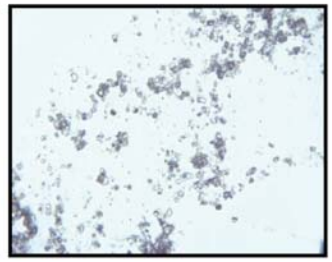


Fig. 2: Positive control A on 8th day



Fig. 3: Positive control B on 22nd day



Fig. 4: Standard control on 22nd day

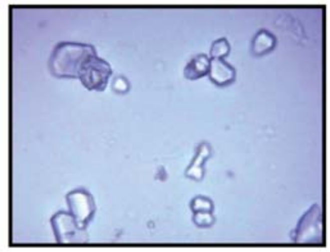


Fig. 5: Test group on 8th day



Fig. 6: Test group on 22nd day

Serum Analysis

It was found that concentration of serum creatinine and urea decreased significantly ($p<0.01$) and a significant reduction was observed in serum calcium ($p<0.001$). No significant change was observed in the serum phosphorus level (Table 3).

Kidney Homogenate Analysis

Calcium and phosphorus were found significantly increased in urolithiatic group. Phosphorus decreased significantly ($p<0.01$) in test group but no significant change was found in calcium (Table 4).

Histopathological Findings of Kidney

In urolithiatic group kidney architecture was found intact with mild increased hypercellularity in Bowman's space. Some tubules have shown irregular homogenous amorphous eosinophilic material. Few uncongested blood vessels were found with increase in mesengial cells and congested capillaries. Dilated and congested blood vessels were observed. Mononuclear inflammatory infiltration was found in interstitium. Changes reverted to the normal level in test group which were almost similar to the findings plain control (Fig. 8-12). On examination with polarized light, intratubular and interstitial crystal deposition was observed in positive controls. But in test and standard groups such type of deposition was not seen (Fig. 7).

Table 2: Effect of the test drug on urinary parameters in "Ethylene glycol-Ammonium chloride induced Urolithiasis" in Rats

Groups	Drug & Dose	Ca (mg/dl)	Creatinine (mg/dl)	P (mg/dl)	Na (mEq/dl)	Cl (mg/dl)	Mg (mg/dl)
Plain control	1 ml of 5% GA	1.568 ± 0.171	0.8798 ± 0.221	63.28 ± 1.53	141.9 ± 10.19	131.6 ± 14.84	1.502 ± 0.28
Positive control A	EG 0.75% and AC1%	5.806 ± 0.973^{a***}	0.1912 ± 0.138^{a*}	73.47 ± 0.86^{a***}	210.2 ± 21.05^{a**}	146.1 ± 6.66	0.371 ± 0.12^{a**}
Positive control B	EG 0.75% and AC1%	8.067 ± 0.206^{a***}	0.1657 ± 0.148^{a*}	70.88 ± 0.32^{a***}	182.8 ± 10.89	147.7 ± 17.17	0.446 ± 0.10^{a**}
Standard control	Cystone 750 mg/kg	1.824 ± $0.251^{e*b*** c***}$	0.8193 ± 0.033^{b*c*}	69.71 ± 0.43^{b*}	142.4 ± 9.36^{b**}	133.8 ± 6.73	1.423 ± 0.12^{b**c*}
Test group	Combination of KC & SK 135 mg/kg	1.834 ± $0.189^{b***e* c***}$	0.8185 ± 0.149	71.04 ± 0.346^{b*}	199.6 ± 27.14^{b**}	134.0 ± 11.39	1.563 ± $0.19^{c**b***}$

*p<0.05, ** p<0.01, ***p<0.001 **a-** compared with plain control, **b-**compared with Positive control A, **c-** compared with Positive control B, **e-** compared with standard control. n=6

Table 3: Effect of the test drug on serum parameters in "Ethylene glycol and Ammonium chloride induced Urolithiasis" in Rats

Groups	Drug & Dose	Ca (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	P (mg/dl)
Plain control	1 ml of 5% GA	8.05 ± 0.415	1.31 ± 0.129	40.26 ± 3.510	2.14 ± 0.320
Positive control A	EG 0.75% and AC1%	10.40 ± 0.654^{a**}	3.05 ± 0.484 ^{a**}	53.65 ± 5.354	3.42 ± 0.453
Positive control B	EG 0.75% and AC1%	9.06 ± 0.888^{b*}	3.02 ± 0.603^{a**}	72.15 ± 9.827^{a**}	3.18 ± 0.355
Standard control	Cystone 750 mg/kg	8.35 ± 0.485^{b*}	1.39 ± 0.047^{b**c**}	42.80 ± 4.354^{c**}	2.41 ± 0.307
Test group	combination of KC & SK 135 mg/kg	7.949 ± 0.376^{b**}	1.404 ± 0.1093^{b**c**}	42.59 ± 2.55^{c**}	2.247 ± 0.214

*p<0.05, ** p<0.01 **a**- compared with Plain control, **b**-compared with Positive control A, **c**- compared with Positive control B. n=6

Table 4: Effect of the test drug on kidney homogenate in "Ethylene glycol Ammonium chloride induced Urolithiasis" in Rats

Groups	Drug and dose	Kidney homogenate analysis	
		Ca (mg/100 gm)	P (mg/100 gm)
Plain control	1 ml of 5% GA	0.834 ± 0.391	2.224 ± 0.348
Positive control A	EG 0.75% and AC1%	3.333 ± 1.037^{a*}	4.290 ± 0.294^{a*}
Positive control B	EG 0.75% and AC1%	2.115 ± 0.583	3.693 ± 0.609
Standard control	Cystone 750 mg/kg	1.054 ± 0.189	2.328 ± 0.318^{b*}
Test group	combination of KC and SK 135 mg/kg	0.9902 ± 0.155	2.058 ± 0.169^{b**}

*p<0.05, **p<0.01

a- compared with Plain_control, **b**-compared with Positive control A, n=6

Discussion

All the rats treated with EG and AC for 7 days developed calcium oxalate crystals in urine which is evident by the presence of crystals in urine microscopy on 8th day (Table 1, Fig. 1-6).Similar findings have been reported by other workers (Rathod et *al*., 2012; Fan *et al*., 1999; Divakar *et al*., 2010). Further it has been

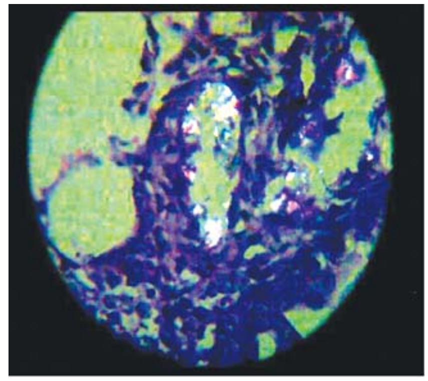


Figure 7: Calcium oxalate crystal deposition in rat kidney tissue seen under polarized microscope (40X) on 8th day

Histopathological reports of rat kidney under light microscopy (400X)

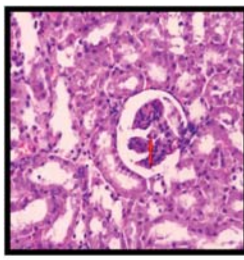
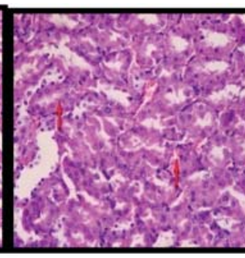
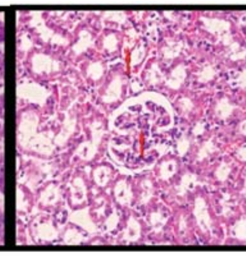
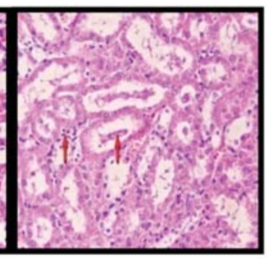
   

Fig. 8: (a) Plain control **8: (b)** Plain control **Fig. 9: (a)** Positive control A **Fig. 9: (b)** Positive control A

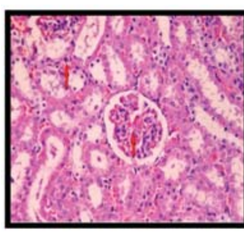
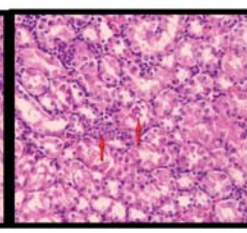
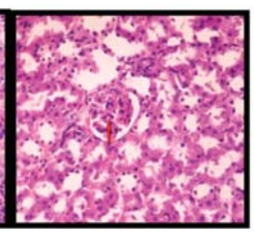
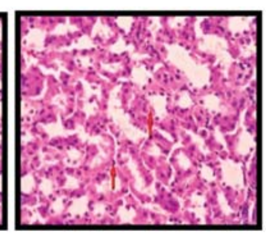
   

Fig. 10: (a) Positive control B: **(b)** Positive control B **Fig. 11: (a)** Standard control **11: (b)** Standard control

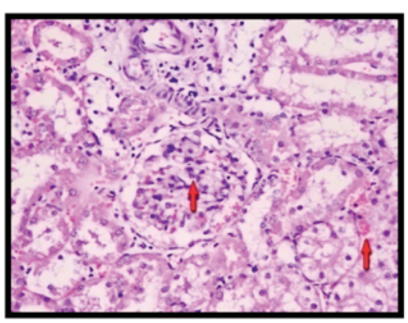
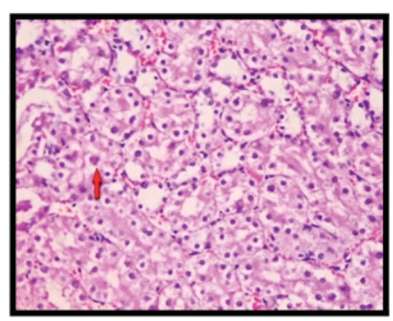
 

Fig. 12: (a) Test group **12: (b) Test group**

reported that EG being oxidized into oxalic acid by non specific dehydrogenase leads to hyperoxalaurea which is considered to be a factor in the causation of lithiasis. EG metabolizes into CaOx monohydrate and produces renal mitochondrial

toxicity similar to clinical CaOx renal calculi (Mc Martin and Wallace, 1984). Renal function was assessed at the end of the study by estimating urinary and serum parameters.

In positive control A (treated with EG and AC for 7 days) and B (left untreated for 14 days after 7 days of treatment with EG and AC) urinary calcium was found increased significantly ($p<0.001$) when compared with plain control. It was also reported in previous studies that EG causes hypercalciuria, hyperphosphaturia and hyperoxalaurea. The increased urinary calcium is a factor favoring the nucleation and precipitation of calcium oxalate from urine and subsequently crystal growth (Lemann *et al*., 1991). Reduction in urinary calcium also reduces super saturation which is main risk factor for stone formation (Dadametirkee *et al*., 2007). Increased excretion of phosphorus has also been reported in stone formers and hyperoxaluric rats ((Rathod *et al*., 2012). After 14 days treatment with test drugs the phosphorus excretion was found to be decreased (although it was not found significant). Urinary sodium was found to be significantly ($p<0.01$) increased in positive control A when compared with plain control, while in positive control B, no significant elevation was observed. A significant reduction ($p<0.01$) was also noted in test and standard groups. The result showed that KC and SK in combined form were comparable with standard drug in reducing super saturation by decreasing the sodium concentration in urine (Table 2).

No significant difference in urinary chloride was observed when test group was compared with positive control groups. Urinary magnesium decreased significantly ($p<0.01$) in positive control A and B. Magnesium is considered an important inhibitor in stone formation ((Divakar *et al.,* 2010) and low level of magnesium has been noted in stone formers as well as stone forming rats (Soundarajan *et al.,* 2006; Rushton *et al.,*1980). In test group a significant elevation ($p<0.001$) in magnesium concentration was observed when compared with positive control A. The results were found even better than the standard drug. The positive control B however showed some elevation in magnesium level but it was less than the test group (Table 2).

Treatment with test drug significantly reduced calcium oxalate in urinary excretion, thus reducing the super saturation of urine. This might be responsible for preventing the formation and dissolving the preformed calcium oxalate type of stones by decreasing calcium and increasing the magnesium level in urine (Akanae *et al.,* 2010). An increase in the concentration of creatinine and urea are the markers of kidney and tubular damage. Glomerular filtration rate is decreased due to injury in kidney tissue. This decrease may also be due to the obstruction in urinary systems because of the stones. Due to this, the waste products, particularly nitrogenous substances may increase (Patel *et al*., 2012). Test drug showed significant decrease in serum creatinine and urea. Serum calcium was observed

to be decreased in test group; although it was non-significant, still it suggested that Test drug improved kidney tissue Ca disruption more than serum Ca.

In histopathological study of the kidneys, cellular derangement, dilated and congested blood vessels, hypercellularity in Bowman's capsule, mononuclear inflammatory infiltration and injured glomerulus were found in positive control groups. While in test drug treated group minimal changes were observed.

The combination of test drugs showed more pronounced effect in comparison to single drug (Humairah *et al.*, 2014), indicating synergistic effect of the two test drugs of the combination. The effect of test drug was found to be comparable to the standard drug Cystone. On inter group comparison between test and standard groups no significant difference was observed, thus it can be concluded that the test and standard drugs are equally effective.

Exposure to high level of oxalate and calcium oxalate crystals has been reported to produce cellular injury mediated by membrane lipid peroxidation through intra cellular reactive oxygen species generation. Therefore, a reduction in renal oxidative stress could be one of the effective measures for the treatment of urolithiasis. It has been reported that flavonoids found in some of the plant drugs decreased oxidative injury in renal tubular cells and calcium oxalate deposition in rat kidney (Jeong *et al.*, 2006; Akanae *et al.*, 2010). SK has been reported to contain ample amount of flavonoid (Shamkuwar *et al.*, 2012). This constituent along with certain other constituents such as saponins and alkaloid in SK and few alkaloids in KC (Parmar, 1997) may have exerted anti oxidant and thereby protective effect (Rathod *et al.*, 2012). Stones are formed due to deficit in the crystallization inhibitory effect of urine and the presence of promoters. When these conditions favor stone formation the anti adherent layer of GAGs acts as a protective barrier against urinary stone disease. If this layer is damaged due to consequence of bacterial attack, a stone nucleus develops leading to full stone formation in urinary tract. At this point the drugs having anti microbial property may be effective (Akanae, 2010). The antimicrobial property of test drugs has already been reported (Aneja *et al.*, 2010; Ahmad et al, 2012) therefore it may be considered as one of the possible mechanisms that the test drug may have evolved as a part of its action. According to Unani concept kidney disease occurs due to change in *mizaj* of kidney or a disturbance in the equilibrium of any of the five *Quwa* (faculties) i.e. *Quwwate Hazima* (digestive power), *Quwwate Jaziba* (absorptive power), *Quwwate Masika* (retentive power) and *Quwwate Dafiah* (evacuating power) and *Quwwate Mumayyizah* (separating power) the kidney is conferred with. In such a condition along with other forms of failure of function removal of viscous and morbid matters become difficult and these matters get entangled in some parts of kidney (Majoosi, 2010). The *hararat* of the kidney dries out these entangled morbid matters, which gradually convert into stone (Razi,

2001; Qamri, 2008; Ibn Sina, 2007). For proper excretion of waste products the *quwa* and the *mizaj* should be in *aitedal* (equilibrium) and *akhlaat* should have the normal viscosity. Therefore the drugs which are relatively less *har* are used to help dissolve the *ghaleez lesdar khilt* (viscid and sticky matter). The two ingredients of test drug are *har* and *yabis* in second degree (Ghani, ynm; Kabiruddin, 2007) and have *mulattif, mufattehe sudad, muhallil* and *mufattite hissat* properties (Ghani, ynm). By virtue of these actions the drugs may have transformed the *ghaleez* matter into *lateef* and made it easy to excrete.

However, since the two components of the test drug have been reported to possess different pharmacological effect, therefore it is likely that the lithotriptic effect was mediated through different mechanisms. Further, the two components appear to produce synergistic effect because the degree of response exerted by the combination was more than the reported effect of the two ingredients individually.

Conclusion

On the basis of results, it can be concluded that test the drug possesses significant lithotriptic effect probably mediated through diverse mechanisms. The ingredients of the test drug have synergistic effect because the combination produced more pronounced effect as compared to the effect reported in respect of its individual ingredients.

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Study of Antifertility Effect of Luk Maghsool *(Laccifer lacca* Kerr) in Albino Rat with Reference to its Antiovulatory and Oestrogenic Activity

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Abstract

Antifertility activity of Luk Maghsool *(Laccifer lacca* Kerr) was evaluated in terms of its antiovulatory and estrogenic/ ant estrogenic activity. In first test the cyclic female Wistar rats were divided into four groups of six each. The animals in plain control received 1ml of 0.5% CMC suspended in distilled water while animals in test group A and B were treated with Luk processed by hot water (LMW) in the dos of 470 mg/kg and 800 mg/kg, respectively. Test group C was given Luk processed with the decoction of *Izkhir* and *Rewand Chini* (LMIR) (800 mg/kg). Second test was carried out in 21days old female rats. Animals were treated with LMW and LMIR (800 mg/kg each) and served as test group A and B, respectively. The animals in standard group were treated with ethinyl estradiole (0.02 mg/kg). In all the experiments, treatment was given once a day orally. In the antiovulatory experiment the treatment continued for 15 days and the vaginal smear were monitored throughout the study. On 16th day animals were sacrificed; ovaries and uteri were weighted; one ovary was subjected to histological studies and other for estimation of cholesterol. Estrogenic nature of Luk was evaluated by noting the times taken for vagina to completely open after 7 days of treatment.

A significant ($p<0.05$) increase in duration of estrus cycle and diestrus phase, with decrease in proestrus ($P<0.01$) and estrus ($P<0.05$) phase was observed in animals treated with LMIR. There was notable decrease in body weight and a significant ($P<0.01$) increase in ovarian tissue cholesterol in test group B and C. Uterine weight was also found to be increased in test groups. Luk also exhibited significant ($P<0.01$) estrogenic activity assessed by immature vaginal opening. It can be concluded that Luk interferes normal ovulation possibly by virtue of its estrogenic effect.

Keywords: *Laccifer lacca*; *Maane' hamal*, Still birth, Implantation, Estrous cycle.

Introduction

The fruits of improvement in different sectors are being engulfed by growing population. Moreover, the increasing number of birth has deleterious effect on health of mother and child, and hinders social and economic upliftment of the family. High parity is also related to increase maternal, prenatal and infant deaths and is associated with various obstetric and gynecological complications and nutritional problems (Dutta, 2006). So there is a need of population control by appropriate measures. For this purpose, the World Health Organization (WHO) has constituted a population control programme, in which traditional medical

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practices have been assigned some important role (Rajandeep *et al.*, 2011).

From the advancement of reproductive biomedicine, several hormonal contraceptive pills have been developed (Rajandeep *et al.*, 2011). Oral contraceptives of the combined type are almost 100 percent effective in preventing pregnancy (Park, 2011). But most of the oral contraceptives are synthetic (hormonal) and have many side effects such as obesity, liver function derangement, liver adenomas, thromboembolism, disturbances in lipid and carbohydrates metabolism (bloating) and therefore are contraindicated in many conditions such as jaundice etc (Dutta, 2006; Park, 2011). So there is a need to develop an effective and safe contraceptive drug from natural sources so as to minimize the side effects associated with commonly used synthetic contraceptive agents. A number of drugs of plant, animal and mineral origin have been described in Unani books to have antifertility property but very few of them have been studied scientifically for their described effect.

Luk *(Laccifer lacca* Kerr) is an important drug of Unani Medicine. Apart from having contraceptive action it is an excellent tonic for liver, blood, stomach and kidney. It also has anti-obesity and deobstruent activities (Ghani, 1971; Baitar, 2003). In Unani system of medicine Luk is being used for anti-obesity effect since centuries. A compound formulation '*Safoofe muhazzil*' in which Luk is included as the chief ingredient, is frequenly prescribed for this purpose. An indigenous preparation (AYUSH-47) having Luk as one of the ingredients in combination with *Saraca indica*, *Areca catechu*, gold and sugar has been claimed to exhibit anti-implantation effect in rabbits (Sughantan and Shanta kumara, 1979). Ghufran et al. (2011*)* studied the Luk for its effects on diet induced hyperlipidemia in albino rats. But the antifertility activity of Luk as a single drug has not been tested so far notwithstanding its powder is advised commonly as a contraceptive by Unani physicians since ages (Ghani, 1971; Khan, 1335 H; Kabiruddin, 2003). Therefore, the present study was designed to study the antifertility effect of Luk in experimental animals. The antiovulatory and oestrogenic activity was determined to evaluate its antifertility potential. Luk has been described to be processed by different methods therefore the two samples of test drug processed by two different methods were used in the study.

Materials and Methods

The present study has been conducted in the department of Ilmul Advia, National Institute of Unani Medicine, Bangalore. The research protocol was submitted to the Institutional Animal Ethics Committee (IAEC) of the institue for ethical clearance before the commencement of the experiment. The study was started after getting the approval of the research protocol by the committee vide its registration number, IAEC/VIII/06/IA.

Test Drug

Luk *(Laccifer lacca* Kerr) was procured with full identity from Indian Institute of Natural Resins and Gums (Formerly Indian Lac Research Institute) Namkum, Ranchi- 834 010, Estd: September 20, 1924 on 23rd May 2012. A voucher specimen of the test drug has been deposited in the museum, Dept. of Ilmul Advia, NIUM, Bangalore, for future reference.

Drug Preparation

Luk was processed according to the method described in Khazainul Advia and Hamdard pharmacopoeia. Half of the sample of Luk was processed by hot water and was named as '*Luk maghsool* with water' (LMW) while the other half was further processed by decoction made of *Izkhir (Andropogan schaenar*) and *Rewand chini;* it was named as '*Luk maghsool* with *Izkhir and Rewand*' (LMIR).

Dosage and Administration

Dosage of the drug for rat was calculated by extrapolating human therapeutic dose i.e. 4 g (Ghani, 1971; Khan 1335 Hijri; Kabiruddin, 2003) by conversion factor of 7 (Freirich et al, 1968) and was found to be 470 mg/kg. A second dose (Miller and Tainter, 1994) was also calculated to determine dose dependent effect of the test drug. It was found to be 800 mg/kg for a rat. The powdered drug suspended in 1ml of 0.5% CMC (Carboxy methyl cellulose), was used for the study.

Experimental Animals

All the tests were carried out on healthy female Wistar rats, weighing 150-250 gm. The animals were procured from Biogen Laboratory Animal Facility (Reg. No. 971/bc/06/CPCSEA), Bangalore. Prior to each experiment, the animals were allowed to get acclimatized for at least one week. They were maintained under standard laboratory condition throughout the experimental period and were provided with standard diet and water *ad libitum*. They were housed in clean polypropylene cages which were placed in a room that was maintained at a temperature 25+2°C and humidity of 45-55%,, with 12 hrs, light-dark cycle. The animal care procedures and experimental protocol were in accordance with the guidelines of CPCSEA.

Experimental Procedures

The vaginal smear of female rats were studied daily microscopically for 15 days. The animals that showed three consecutive normal estrus cycles were selected for the evaluation of antiovulatory activity.

Test for Antiovulatory Activity

This test was carried out by the method of Koneri *et al* (2006) and Alia *et al* (2012). The selected rats were divided into four groups of six animals each i.e. plain control, test group A, B and C. The animals in plain control received 1ml of 0.5% CMC suspended in distilled water while those in Test group A and B were treated with powdered LMW in the dose of 470 mg/kg and 800 mg/kg, respectively. The animals in Test group C were given powdered LMIR in the dose of 800 mg/kg. The treatment was started in estrus phase with daily oral dosing for 15 days. Vaginal smear from each animal was continued to be observed every morning between 9 and 10 a.m. for the next 15 days (Marconde, 2002; Bandopadhaya, 2010). Three types of cells were recognized: round and nucleated ones were epithelial cells; irregular ones without nucleus were the cornified cells; and the little round ones were the leucocytes. Their mutual proportion was used for the determination of the estrus cycle phases (Fig. 1, 2, 3 &4). On the 16^{th} day, 24 hrs after the last treatment all the animals were sacrificed under thiopentone anaesthesia (50 mg/kg, IP). The ovaries and uteri were carefully dissected out, freed from extra deposition of fat and weighed. One of the two cleaned and weighed ovaries from each rat was then processed for the tissue cholesterol estimation. Cholesterol was estimated using Total cholesterol ppt. set. The results were expressed in microgram/milligram of ovary (Kaur and Dhanju, 2005). Histological study of ovary of each rat was carried out by a pathologist at Prakash Diagnostic Laboratory, Bangalore. The sections of the ovaries were observed for the enumeration of graafian follicles and corpora lutea.

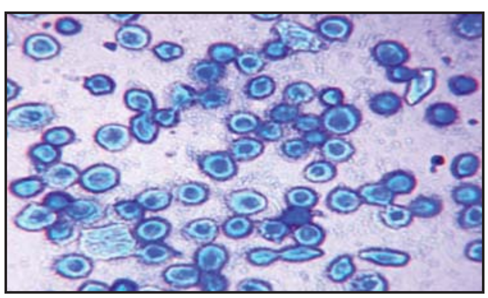


Fig. 1: Proestrus

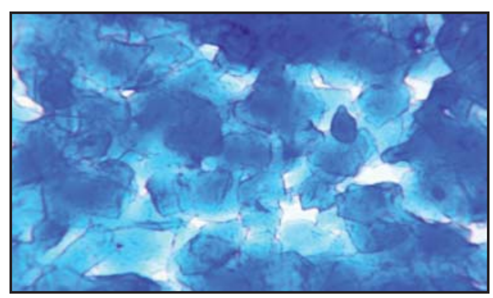


Fig. 2: Estrus

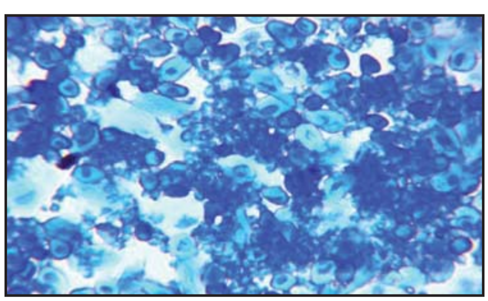


Fig. 3: Metestrus

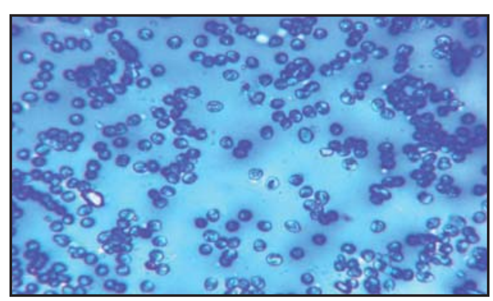


Fig. 4: Diestrus

Test for Estrogenic/Anti-estrogenic Activity

This test was conducted by the method of Gupta (2009). Immature female rats of 21 days weighing 25-35g were divided into four groups of six animals each. Group I served as plain control and was administered 1ml/kg of 0.5% CMC suspended in distilled water orally. Group IV was administered ethinyl estradiol 0.02 mg/kg which served as standard group. While group II and III were treated with powdered LMW and powdered LMIR in the dose of 800 mg/kg and served as test group A and B, respectively. All the above treatments were given orally once a day for 7 days. The vaginal opening was noted every morning between 9-10 a.m. The complete vaginal opening was observed as the sign of estrogenic activity.

Statistical Analysis

The data was analyzed using Graph pad software. ANOVA one-way with Post hoc Tukey-Kramer comparison test was applied for the parameters which passed normality test, otherwise Kruskal Wallis test with Dunn's comparison was used.

Observations and Results

Effects of Luk on Ovulation by Vaginal Smear Observation

The number of days spent in proestrus phase by test group A and B were not significantly different from plain control but it was found reduced significantly ($P<0.01$) in test group C. The number of days spent by rats in metestrus phase increased significantly ($p<0.01$) in test group A in comparison with test group B, C and plain control group but no significant difference was observed in test group B and C when compared with plain control and to each other. Test group A and B showed no significant difference in duration of diestrus phase from plain control. But test group C exhibited significant ($P<0.05$) increase in number of days from plain control. Test group B and C exhibited a significant ($P<0.01$) increase as compared to test group A, while no significant difference was observed among test group B and C on comparison with each other. No significant difference was observed in test group A and B in the duration of estrous cycle when compared with plain control, while Test group C showed significant ($P<0.05$) difference. No significant difference was observed among the test groups (Table 1).

Rats' Body Weight, Uterine Weight, Ovarian Weight

The mean difference weight gain by the rats, before and after the treatment, in the plain control group was calculated as 28.67±2.43, which was significantly ($P<0.01$) different from those in Test group A, B and C were it was found to be 12.17±2.27 gm, 6.17±1.50 gm and12.67±2.43 gm, respectively (Table 2). A notable

Table 1: Effect of *Luk (Laccifer lacca*) on the duration of the different phases of estrus cycle in rats

Treat-ment	Dose (mg/kg)	Number of days spent in each phase				
		Proestrus	Estrus	Metestrus	Diestrus	Duration of estrus cycle
Plain	CMC1ml	3.67±0.21	3.67±0.21	4.00±0.00	4.50±0.55	4.11±0.70
Test A	LMW470 mg	2.50±0.43	2.67±0.21*	6.50±0.43**,aa	3.33±0.21	4.70±0.14
Test B	LMW800 mg	3.17±0.17	3.17±0.17	3.50±0.43	5.50±0.62aa	4.39±0.49
Test C	LMIR800 mg	2.00±0.36**	2.67±0.21*	4.17±0.31	6.17±0.48*,aa	4.72±0.53*

LMW=*Luk maghsool* with water, LMIR=*Luk maghsool* with *Izkhar Rewand*. Data presented: Mean ± SEM; n = 6 in each group; *P<0.05, **P<0.01 when compared with plain control. aa P<0.01when compared test group B and C with A.

Table 2: Effect of *Luk (Laccifer lacca*) on the body weight of rats

Groups	Treatment	Dose	Initial body weight (gm)	Final body weight (gm)	Mean wt. Difference (gm)
Plain Control	CMC (0.5%)	1ml/kg	182.33±8.44	211.00±10.76	28.67±2.43
Test Group A	LMW	470 mg/kg	176.00±4.00	186.17±5.49	12.17 ± 2.27**
Test Group B	LMW	800 mg/kg	180.50±11.22	186.67±10.66	6.17±1.50**
Test Group C	LMIR	800 mg/kg,	191.17±7.41	203.50±5.65	12.67±2.43**

LMW=*Luk maghsool* with water, LMIR=*Luk maghsool* with *Izkhar Rewand*. Data presented: Mean ± SEM; n = 6 in each group; **P<0.01when compared with plain control.

increase in the mean weight of uterus was noted in Test group A, B and C in comparison with the plain control, however, not yet, statistically significant. A non significant difference in mean ovarian weight of rats was observed in test groups A, B and C in comparison to plain control. Also no significant difference was observed in test groups when compared with each other. (Table 3)

Ovarian Cholesterol Level

The mean cholesterol level in the ovarian tissue in test group A was 8.88±0.90ìg/mg which was statically insignificant in comparison to plain control (9.59±0.73ìg/mg). An increase (P<0.01), however was observed in Test groups B and C where the level of cholesterol was found to be 15.31±1.13ìg/mg and 15.19±0.84ìg/mg, respectively on comparison with plain control. No significant difference was found between test group B and C (Table 3).

Table 3: Effect of *Luk (Laccifer lacca*) on the weights of ovaries, uteri and ovarian tissue cholesterol in rats

Groups	Treatment	Dose	Uterine weight (mg/100gm of rat)	Ovarian weight (mg/100gm of rat)	Ovarian cholesterol (µg/mg of ovary)
Plain Control	CMC (0.5%)	1ml/kg	135.23±9.44	15.54±1.06	9.59±0.73
Test Group A	LMW	470 mg/kg	164.00±21.07	15.42±1.50	8.88±0.90
Test Group B	LMW	800 mg/kg	201.88±28.03	13.62±0.79	15.31±1.13**,aa
Test Group C	LMIR	800 mg/kg	179.02±20.60	15.19±1.59	15.19±0.84**,aa

LMW=*Luk ma*ghsool with water, LMIR=*Luk maghsool* with *Izkhar Rewand*. Data presented: Mean ±SEM; n = 6 in each group; **P<0.01 when compared with plain control and aa P<0. 01 when compared with Test group A

Histological Study of Rat Ovaries

The ovarian sections of plain control group (Fig. 5) showed the cortex with medulla. The medulla showed congested vascular spaces with spindle shaped cells. The cortex showed primordial follicles, primary follicles, secondary follicles, graafian follicles in all stages of maturation along with corpora lutea in the stroma. The follicle showed ovum and its nucleus surrounded by follicular cells. In test group A and B (Fig. 6 & 7) the medulla showed congested vascular spaces and spindle

Histological study of rat ovaries

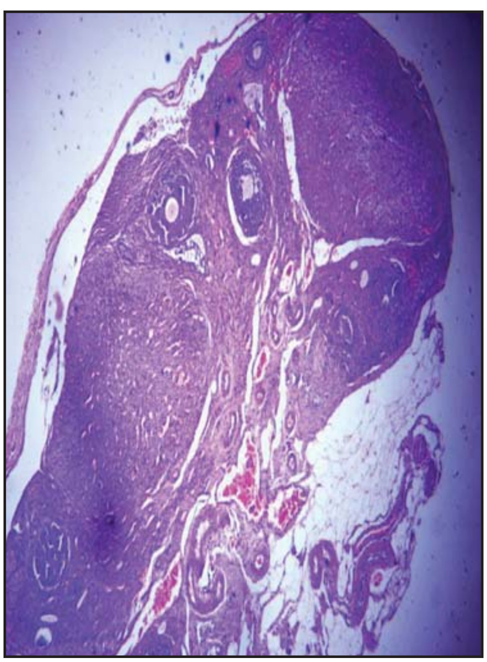


Fig.5: Plain control group (HPE4X)

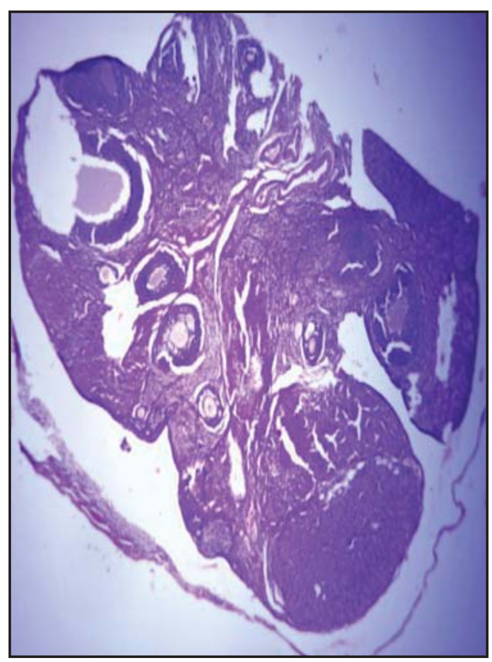


Fig.6: Test group A (HPE4X)

shaped cells. The cortex showed increase number of primordial follicles, primary follicles, secondary follicles, graafian follicles in all stages of maturation. Most of the granulosa cells and theca near the follicular antrum showed degenerative changes. Corpora lutea showed signs of fibrosis and appeared small. In test group C (Fig. 8) all the findings were similar as that observed in test group A and B except that, it showed arrested ovum in follicles at various stages of development.

Effect of Luk on Vaginal Opening

The mean time taken for complete vaginal opening by rats in plain control was 25.5±0.43 days; test group A took 6.83±0.30 days; test group B 21.17±0.30 days,

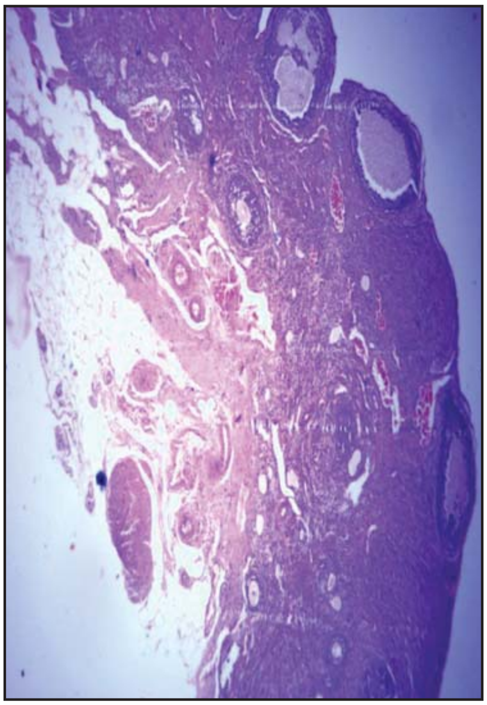


Fig. 7: Test group B (HPE4X)

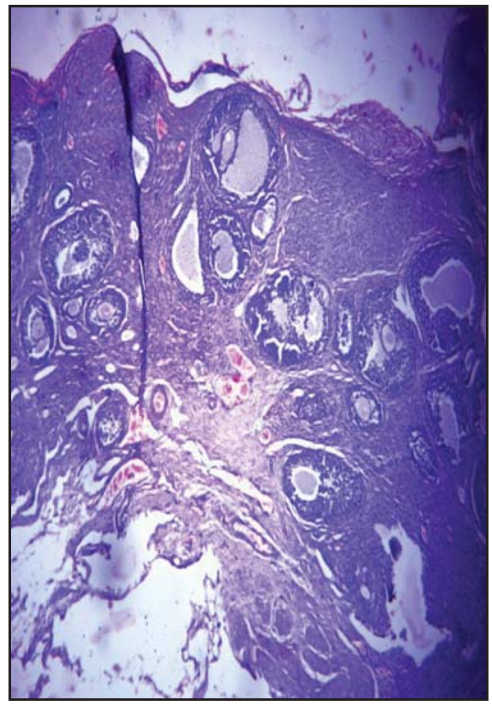


Fig. 8: Test group C (HPE4X)



Fig. 9: Arrow demarcating closed vagina before the treatment



Fig. 10: Arrow demarcating open vagina after treatment

while standard group took 6.50±0.22 days. All the test and standard groups showed significant ($P< 0.01$) reduction in when compared with plain control. Statistically no significant difference was found between test group A and standard group while very significant ($P<0.01$) difference was found when test group A and standard group were compared with test group B (Fig. 9 &10, Table 4).

Table 4: Effect of *Luk (Laccifer lacca*) on vaginal opening

Groups	Treatment	Dose	Duration of treatment	Vaginal opening on 7th day	No. of days taken for complete vaginal opening
Plain Control	CMC (0.5%)	1ml/kg	7days	Not open	25.5±0.43
Test Group A	LMW	800 mg/kg	7days	Open	$6.83±0.30^{**,aa}$
Test Group B	LMIR	800 mg/kg	7days	Not open	21.17±0.30**
Standard group	Ethinyl estradiol	0.02mg/kg	7days	Open	$6.50±0.22^{**,aa}$

LMW=*Luk maghsool* with water, LMIR=*Luk maghsool* with *Izkhar Rewand*. Data presented: Mean + SEM; n = 6 in each group; **$P<0.01$ when compared with plain control. aa$P<0.01$ when compared with Test group B.

Discussion

Luk was processed in two different ways i.e. one with water and other with the decoction of *Izkhir* and *Rewand chini* and named as LMW and LMIR, respectively. The LMW was used in doses of 470 mg/kg and 800 mg/kg, but since drug exhibited dose depended response therefore LMIR was used only at higher dose.

During estrus cycle many physiological, biochemical, morphological and histological changes occur in ovaries. Physiologically in estrus cycle follicular growth and ovulation are regulated by endocrine (FSH, LH, and Prolactin) and ovarian hormones (progesterone estrogen and androgen). Imbalance in these hormones leads to irregularity in the ovarian functions and duration of estrus cycle (Prakash and Mathur, 1979; Shivaligappa *et al*., 2002; Circosta *et al*., 2001). In this study results showed that Luk is disrupting the hormonal balance which is necessary for normal functioning of ovaries and for maintaining regular estrus cycle. With the increase in dose, diestrus phase is getting prolonged and further by LMIR proestrus and estrus is shortened. Prolonged diestrus phase indicates prolongation of luteal phase i.e. corpora lutea are getting retained for longer duration and thereby prolonging the cycle and causing a disturbance in ovulation. This suggests that effect of Luk depends on the dose and the method it was

processed with. Similar findings have also been reported in other studies (Gbotolorum et al., 2008; Sheeja *et al*., 2009). Estrogen and progesterone are steroid hormones. They are derivatives of cholesterol that are synthesized most prominently by the adrenal gland and gonads i.e. cholesterol is the precursor for the steroidogenesis of ovarian endocrine tissue (Guyton and Hall, 2006). The increase in cholesterol level of the groups treated with high dose of LMW and LMIR indicated non utilization of cholesterol towards biosynthesis of hormones in ovaries. The results are in conformity with the findings report by Sheeja *et al.* (2009) and Bandyopadhyay (2010).

During the ovarian cycle some proliferative changes take place in the uterus under the influence of estrogen as a result of which uterine weight increases (Dhar, 1995). In the present study an increase in uterine weight in test groups indicated the estrogenic activity of test drug. The findings of this study were further assisted by observed follicles and corpora lutea in ovarian histology, as both higher dose of LMW and LMIR showed increased number of follicles in various stages of development and retained corpora lutea showing signs of fibrosis, whereas in LMIR treated animals arrested ovum in follicles at various stages of development were also seen. These changes can be attributed to estrogen like substances in the drug as estrogen is essential for maturation and differentiation of ovarian follicles. But imbalance in endogenous steroid, protein and hormone has resulted in retained corpora lutea. Further corpora lutea showed central fibrous tissue formation in the ovarian stroma indicating increased survival of corpora lutea and subsequent prolongation of the diestrus phase (Sheeja *et al*., 2009).

According to Unani literature ovaries possess the reserve of *ratubat ghariziya* which is precursor of *maddae manwiya* (primordial follicles). Sufficient *hararat* and *ratubat* is required in ovaries for the production of *maddae manwiya* and its subsequent transformation in to mature *mani* (ova). Imbalance in the above *kaifyaat* may undermine the function of ovaries which may result in production of defective ovum. Luk is a drug having *har yabis* temperament in second or third degree. So in early stages *hararat* acts on *ratubat ghariziya* of the ovaries, resulting in stimulation of ovarian function but as the *ratubat ghariziya* starts disappearing after sometime, the *yabis* property of drug further leads to *tajfeef*. Once *ratubat* gets exhausted; the ovaries lose their normal physiological function. The production of *mani* gets hampered and the *maddae manwiya* (primordial follicles) fails to mature in *mani* (fully formed ova) (Majoosi, 1889; Ibn Rushd, 1987; Tabri, 2010; Razi, 1991). This concept is evidenced by our findings that in the beginning of treatment estrus phase became more obvious while at later stage diestrus predominated all phases of estrus cycle. Further the histological findings also demonstrated many arrested ova in various stages of developing/maturing follicles. Therefore it can be concluded that Luk has disrupted the production of ovum, maturation of ovum and ovulation probably by alteration of temperament of ovaries.

Luk was further evaluated for estrogenic activity by noting the time taken by the immature rats for complete vaginal opening. It was found that rats treated with LMW took almost as much time as by the Ethinyl estradiole treated group revealing that drug possesses potent estrogenic effect. Both the test groups i.e. LMW and LMIR exhibited significant ($P< 0.01$) reduction in number of days for complete vaginal opening in comparison to plain control. This suggests that Luk possesses estrogenic activity. As it is well known facts that balance is needed between estrogen and progesterone for normal estrus cycle so the possible mechanism by which drug produced antiovulatory effect may be the estrogen like effect.

In test groups notable decrease in body weight was found in comparison to plain control indicating that it can overcome weight gain problem faced by the users of synthetic contraceptives. It also justified the anti-obesity effect of Luk (Baitar, 2003; Kabiruddin, ynm).

The better response produced by LMIR may be attributed to the specific method of its processing as the treatment with the decoction of *Izkhir* and *Rewand chini* has been described to increase the absorbability and thereby the response of the drug (Baitar, 2003).

Conclusion

It can be concluded that Luk possesses significant antifertility effect. This effect may be attributed mainly to its *har yabis* (hot & dry) temperament and the estrogenic property.

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Hepatoprotective Activity of a Unani Polyherbal Formulation "Kabideen" in Paracetamol Induced Liver Toxicity in Rats

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Abstract

Kabideen (Syrup) is a Unani polyherbal formulation manufactured and marketed by a reputed Unani Pharmaceutical establishment Dawakhana Tibbiya College, Aligarh Muslim University Aligarh, India. This preparation is being prescribed by the physicians of traditional medicines in the management of liver disorders since last many years. Although the reports of physicians suggest that it is clinically very effective hepatoprotective drug, but scientific studies have not been conducted so far on this product to validate the claims of Unani physicians. Therefore the present study was designed to investigate the self prepared (SP) and market sample (MS) of Kabideen for hepatoprotective activity against paracetamol induced liver damage in albino rats of either sex at a dose of 5.25 ml/kg body weight. Various biochemical parameters of liver function including serum total bilirubin, total protein, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total cholesterol and lipid peroxidation were measured to assess the effect of the test drug on paracetamol induced hepatic damage. The histopathological study of liver tissue was also conducted. Results of the study revealed elevated level of marker enzymes in the animals treated with paracetamol (750 mg/kg intraperitoneally), indicating severe hepatic damage, whereas significant reduction in the serum markers were seen in the animals treated with two samples of Kabideen indicating significant hepatoprotective effect possessed by the test drug. However, the effect produced by the self prepared sample was more striking.

Keywords: Kabideen, Hepatoprotective activity, Paracetamol damage.

Introduction

Liver has a pivotal role in regulation of physiological processes. It is involved in several biochemical pathways related to growth, nutrient supply, metabolism, secretion and storage. Liver diseases are mainly caused by chemical intoxicants (certain antibiotics, carbontetrachloride, chemotherapeutics, peroxidised oil, aflatoxins, excess consumption of alcohol, high doses of paracetamol and infections). Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity (Doreswamy and Sharma, 1944 and Handa *et al.*, 1989). Only some of the plants with hepatoprotective property and few formulations used in Unani medicines have been evaluated pharmacologically for their hepatoprotective and associated effect on experimental and clinical models (Farooq *et al.,* 1997; Katuria and Singh, 1974). A number of polyherbal formulations both pharmacopoeial and non pharmacopoeial are available in the markets which

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are being used successfully to treat liver diseases, but most of them have still not been scientifically evaluated for the effects these are being used for. Kabideen (syrup) is a proprietary preparation of Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh; it has been described to be effective in different types of liver disorders. It comprises of 21 ingredients (Table 1) of Unani herbal drugs that are attributed to have either hepatoprotective effect or are able to induce a response that complement the hepatoprotection directly or indirectly. According to the literatures of Unani medicine most of the ingredients of this formulation are used as Muhallil (anti-inflammatory), Muffatteh (deobstruent), Mudir (diuretic) and Muhafiz kabid (hepatoprotective), and are prescribed effectively in liver diseases by the physicians of traditional medicine.

Table 1: Ingredients of Kabideen

S.No.	Unani Name	Botanical Name	Part used	Quantity
1	Biranjasif	*Achellia millefolium*	Top of Flowers	10 gm
2	Barg-e-Shahattara	*Fumaria officinalis*	Leaves	6 gm
3	Barg-e-Kasaundi	*Cassia occidentalis*	Leaves	6 gm
4	Tukhm-e Kasni	*Cichorium intybus*	Seeds and root	10 gm
5	Tukhm-e-Bathua	*Chenopodium alba*	Seeds	6 gm
6	Tukhm-e-Kasoos	*Cuscuta reflexa*	Seeds	6 gm
7	Tukhm-e-Khayarein	*C.sativa and c.melo*	Seeds	6 gm
8	Mako	*Solanum nigrum*	Fruits	10 gm
9	Rewand Chini	*Rheum emodi*	Rhizomes	7 gm
10	Sumbullut Teeb	*Nordostachys jatamansi*	Rhizomes	6 gm
11	Ood Hindi	*Aquillaria agallocha*	Roots	6 gm
12	Narmusk	*Ocrocorpus longifolius*	Buds	6 gm
13	Satar Farsi	*Zataria multiflora*	Leaves	6 gm
14	Ushba	*Smilax regelli*	Roots	6 gm
15	Khulanjan	*Alpinia galanga*	Roots	6 gm
16	Chiraita Shireen	*Swertia chirata*	Leaves	3 gm
17	Gul-Surkh	*Rosa demascena*	Flowers	3 gm
18	Gul-e- Nilofar	*Nymphaea alba*	Flowers	6 gm
19	Gul-e-Tisoo	*Butea frondosa*	Flowers	10 gm
20	Gul-e-Ghafis	*Agrimonia eupatorium*	Flowers	10 gm
21	Bekh-e-Kasni	*Cichorium intybus*	Roots	6 gm

In view of the above therefore the present study was designed to investigate the hepatoprotective effect of Kabideen against paracetamol induced hepatic damage in experimental model. Paracetamol (acetaminophen) is a widely used antipyretic and mild analgesic drug which produces acute liver damage if overdoses are consumed. It is mainly metabolized in liver to excreteable glucuronide and sulphate conjugates (Jollow *et al*., 1974 and Wong *et al*., 1981). Two samples of Kabideen were used during the study. One sample was prepared by us (self prepared, henceforth SP) and other was procured from the company that prepares it (Market sample, henceforth MS).

Materials and Method

Collection and Authentication of Plant

The ingredients of Kabideen were procured from local market of Aligarh. The samples were identified and authenticated by NISCAIR, New Delhi and the Pharmacognosy section of the Department of Ilmul Advia, Aligarh Muslim University. The ingredients were used to prepare the sample of Kabideen (SP).

Kabideen syrup (MS) was procured from Dawakhan Tibbiya College, Aligarh Muslim University, Aligarh.

Method of Preparation of Kabideen Syrup (SP)

The decoction of the ingredients was poured into a tin-coated vessel and added 2.5 parts of sugar, and then the vessel was kept on low fire and waited till it attained the required consistency (Anonymous, 2006).

Experimental Animals

Wistar albino rats of either sex weighing 150- 250 g were used for the study. The animals were procured from Central Drug Research Institute (CDRI), Lucknow. The animals were placed in polypropylene cages with paddy husk as bedding. They were fed on standard diet and water *ad libitum* and housed at a temperature of 24±2 ^{0}C and relative humidity of 30-70%.

Experimental Design

All the animals were divided into five groups consisting of 6 animals each. They received the treatment as follows:

Group I   received normal saline in the dose of 1ml/kg/p.o for 7 days and served as control

Group II treated with Paracetamol in the dose of 750 mg/kg i.p. on 7th day and served as model for paracetamol toxicity and it was

Group III treated with SP (5.25ml/kg/day) for 7 days and paracetamol (750mg/kg i.p.) on 7th day.

Group IV received MS (5.25ml/kg/day) for 7 days and paracetamol (750mg/kg i.p.) on 7th day.

Group V received Silymarin (50mg/kg/day,p.o) for 7 days and paracetamol (750mg/kg i.p.) on 7th day.

All the animals were sacrificed after 36 hours and biochemical tests on blood sample and histopathological studies on liver tissues were performed (Vivek *et al*., 1994).

Biochemical Estimation

After sacrificing the animals, the blood was collected and centrifuged at 7000 rpm for 15 minutes and stored at 4^{0}C. AST (Moss, 1994), ALT (Moss, 1994), ALP (Kaplan and Lavernel, 1983), Bilirubin (Malloy and Evelyn, 1937), Total protein (Kingsley, 1939), Total cholesterol (Abell *et al*., 1952) and lipid peroxidation (Ohkawa *et al*., 1979) were estimated in serum.

Histopathological examination: The liver of all the animals was removed and preserved in 10% formalin solution for histopathalogical investigations (Luna, 1966).

Statistical Analysis: All the data were expressed as Mean ± S.E.M and analyzed statistically using one way ANOVA and compared with respective control group by graph pad instat. A value of $P<0.05$ was considered significant.

Results

Biochemical

Effect of the two samples of Kabideen i.e. SP and MS on paracetamol induced liver injury in rats with reference to biochemical changes in serum and lipid peroxidation are given in Table 2 and 3. Histological profile of liver tissue is depicted in Figure 1, 2, 3, 4 and 5. Blood samples of paracetamol treated animals collected at the end of the treatment showed significant increase in the serum level of total bilirubin, alanine amino transferase, aspartate amino transferase, alkaline phosphatase, lipid peroxidation and cholesterol as compared to the normal control group, while the total protein level decreased reflecting liver injury caused by paracetamol; whereas blood samples from the animals treated with SP and MS

Table 2: Effect of test drug Kabideen (SP and MS) on paracetamol induced toxicity

Group	S. ALT/SGPT (Units/ml) (Mean ± SE)	S.AST/SGOT (Units/ml) (Mean ± SE)	Serum Bilirubin (mg/dl) (Mean ± SE)	S.Alk. Phosphatase (Unit/dl) (Mean ± SE)	Total Protein (gm/100 ml) (Mean ± SE)	Total cholesterol
Plain control	26.77± 0.74 z*a*b*	51.06± 0.94 z*a*b*	0.74±0.02 z*a*b*	65.48±1.76 z*a*b*	6.01±0.12 z*a*b*	78.46±2.31 z*a*b*
PCM 750 mg/kg	91.71±1.31 x*	91.50±2.06 x*	2.23±0.07 x*	170.2±0.94 x*	4.40±0.01 x*	162.13±2.31 x*
SP 5.25 ml/kg	74.40±1.06 Y*	78.32±2.13 Y*	1.83±0.03 Y*	134.51±2.31 Y*	4.88±0.09 Y*	149.93±0.57 Y*
MS 5.25ml/kg	90.57±0.83 Y*	85.99±1.60 Y*	2.01±0.03 Y*	157.22±0.09 Y*	4.41±0.02 Y*	156.26±3.14 Y*
Silymarin 50 mg/kg	62.76±1.26 y*	64.02±1.14 y*	0.93±0.14 y*	95.97±9.02 y*	5.12±0.10 y*	98.78±3.29 y*

n=6 *P< 0.001

x = against plain control
y= against PCM (750mg/kg)
z= against standard (silymarin 50 mglkg)
a= against SP (5.25 ml/kg)
b= against MS (5.25 ml/kg)

Table 3: Effect of Kabideen on Lipid Peroxidation in Paracetamol induced hepatic damage

Groups	Lipid peroxidation (n mole of MDA/mg of protein) (Mean ± SE)
Plain control	1.99 ± 0.20 z*a*b*
PCM 750mg/kg	7.38 ± 1.70 x*
SP 5.25 ml/kg + PCM 750 mg/kg	2.64 ± 0.10y*
MS 5.25 ml/kg + PCM 750 mg/kg	3.10 ± 0.07y*
Silymarin 50 mg/kg + PCM 750 mg/kg	2.53± 0.29y*

n=6 *P< 0.001

x = against plain control

y = against PCM (750mg/kg)

z = against standard (Silymarin 50mglkg)

a = against SP (5.25ml/kg)

b = against MS (5.25ml/kg)

at the dose 5.25 ml/kg showed significant decrease in the level of serum marker enzymes while a significant increase in the total protein was observed. The findings suggested that the test drugs protected the hepatic cells from the likely injury of paracetamol. The values in respect of SP were comparable to the values determined in the group treated with standard drug (Silymarin 50 mg/kg/day). Further, the response of SP was comparatively better than the MS.

Histopatological

Fig 1. shows normal hepatic architecture with single plate of heptic cords and normal hepatocytes with central vesicular nucleus and prominent nucleoli. Fig 2. PCM treated liver showing condensation of nuclear chromatin, foci of necrosis around congested and dilated central vein with nuclear pyknosis and cytoplasmic eosinophilia with cloudy swelling. Fig 3. SP treated Group showing mild microvesicular degeration of hepatocytes around central vein with maintained central vein and hepatic cords. Fig 4 MS treated Group showing moderately bilated and congested central vein & portal vein with marked interportal and periportal fibrosis. Fig 5. Silymarin treated liver showing mildly congested sinusoids with centrilobular necrosis and mild fibrosis.

Histology

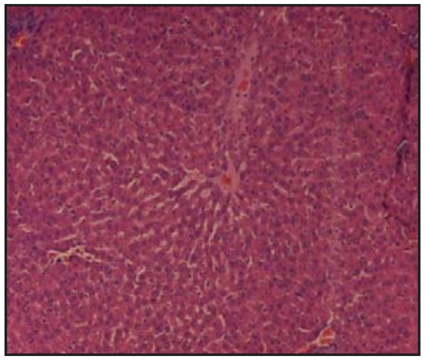


Figure 1. Normal

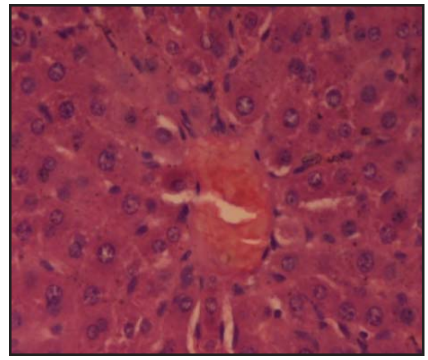


Figure 2. (Paracetamol treated)

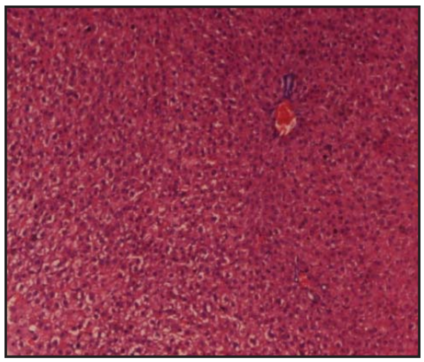


Figure 3. (SP treated)

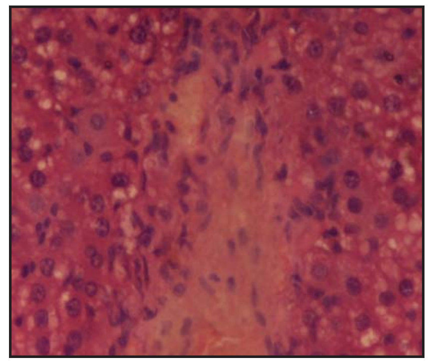


Figure 4. (MS treated)

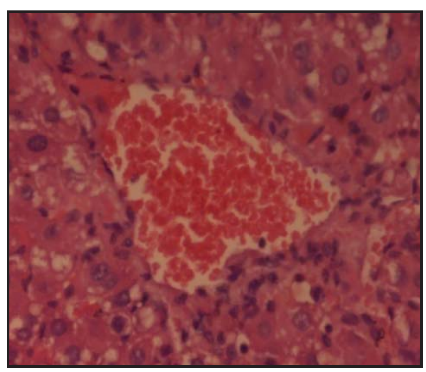


Figure 5. (Silymarin treated)

Discussion

Paracetamol induced hepatic injury is an experimental model widely used for the screening of hepatoprotective drugs. Paracetamol undergoes a biotransformation by hepatic microsomal cytochrome p450 to produce trichloromethyl free radicals. These hepatotoxic metabolites can react with protein and lipid in the membrane of cells or organelles leading to necrosis of hepatocytes. As a result of hepatic injury, the altered permeability of the membrane causes the enzymes from the

cells to be released into the circulation. The magnitude of hepatic damage is usually assessed by measuring the level of released cytosolic transaminases including ALT and AST in the circulation. The rise in the serum levels of ALP, AST and ALT as observed in the present study could be attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and are released into circulation after cellular damage. Other researchers have reported that increased level of AST, ALT, ALP, bilirubin, cholesterol and decreased level of protein are due to PCM hepatotoxicity. The increase in level of serum bilirubin is an index of degree of jaundice. It could possibly be the result of increased production, decreased uptake by liver, decreased conjugation, decreased secretion from liver. Table 2,3 represents that the administration of paracetamol significantly increased the levels of AST, ALT, ALP, cholesterol, lipid peroxidation and bilirubin, and decreased the total protein level due to damaged structural integrity of the liver because these are cytoplasmic in location and are released into circulation after cellular damage. Kabideen prevented the paracetamol induced perturbations in the activities of AST, ALT, ALP, cholesterol, lipid peroxidation, total protein and bilirubin. The findings of the study were further complemented by histopathological observations (Fig 1-5) that suggested that the structural integrity of the liver tissue was maintained to a great extent in the groups treated with Kabideen. As compared to the control group, paracetamol intoxicated rats showed mild inflammatory cell infiltration and fatty changes, but the administration of Kabideen for 5 days attenuated these histopathological changes. Since paracetamol induces injury by stimulating the formation of free redicals therefore the hepatoprotective effect appears to be mediated through antioxidant property of Kabideen.

Conclusion

In the light of the findings it can be concluded that the two samples of Kabideen possesses significant hepatoprotective effect in experimentally induced liver injury. However the effect produced by the SP was more prominent as compared to MS.

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A Comparative Clinical Study of Kabdeen and Liv52 in *Warm-e-Kabid Haad Vairoosi* (Acute Viral Hepatitis-B)

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Abstract

Warm-e-Kabid Haad Vairoosi (Acute Viral Hepatitis) is usually caused by one or more of the five viral agents. Among all hepatitis viruses, Hepatitis-B virus is one of the most grievous viral infection which may culminate in liver cirrhosis, carcinoma of the liver or fulminant hepatitis leading to death. In Unani Medicine, plants as a whole or their parts are extensively used for the cure of liver derangements. They are likely to be effective and much safer. In the present study, a comparative therapeutic evaluation of kabdeen and Liv-52 in 20 patients of Warm-e-Kabid Vairoosi (viral hepatitis B) was done. The study shows that the Unani Test Formulation 'Kabdeen' produces significant improvement in cases of Hepatitis-B. Comparison of Kabdeen with the standard agent Liv-52 shows that in the most parameters, the latter is more effective while in some parameters Kabdeen is more effective.

Keywords: Acute Viral Hepatitis-B, Kabdeen, Liv-52, Hepatoprotective activity, Antiviral activity.

Introduction

Liver is the largest exocrine gland of the body which performs a wide variety of functions like nutrients metabolism, protein synthesis, metabolism and excretion of drugs, alcohol, bilirubin and hormones. Apart from storage of vitamin A, B_{12} and Iron it is actively engaged in blood coagulation mechanism of the body also. It is also the only organ next to the pituitary gland in the body which receives the dual blood supply, one from the hepatic artery and another from the portal vein. Therefore, it is more vulnerable to systemic as well as gastrointestinal infections and toxins. Hence it can be insulted in one way or the other. Liver can be insulted in several ways out of which viral infection is the most common cause. Almost all cases of acute viral hepatitis are caused by the five viral agents, viz. Hepatitis A Virus (HAV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Hepatitis D Virus (HDV) and Hepatitis E Virus (HEV). All these human hepatitis viruses are RNA virus except for the hepatitis B which is a DNA virus. (Braund Wald et al. 2008, Golwalla, *et al.*, 2000, Macleod, 2006,). In general the clinical features of all the types of viral hepatitis are similar and can be divided broadly into three phases i.e. Prodromal phase, Icteric phase and Convalescent phase. (Siddiqui, *et al.*, 2005).

In this study authors have focused over the acute viral hepatitis caused by hepatitis B virus infection (HBV) because, firstly this infection enters in the chronic phase which very often cause Cirrhosis of Liver, Carcinoma of Liver and Fulminant

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hepatitis which frequently ends in death. Secondly the diagnosis of hepatitis B viral infection was easy as compared to the other viral infections because the anti-sera of other viral infections were very costly and were beyond the reach of our limited budgeting resources.

Materials and Methods

The study was conducted on 30 cases of *Warm-e-kabid Vairoosi* (viral hepatitis) who attended the outdoor and indoor sections of department of Moalijat, Ajmal Khan Tibbiya College Hospital, Aligarh Muslim University, Aligarh in which the presenting feature was jaundice due to acute viral hepatitis. In this research work only the clinical trial on the patients suffering from viral hepatitis B is presented however, Kabdeen, a well- known Unani formulation used in liver disorders was studied for effect on Hepatitis-B. Liv-52 was used as Control Treatment. In the present study 20 patients were selected randomly suffering from viral hepatitis due to Hepatitis-B virus infection confirmed by serological test. The patients were divided into two groups of 10 each. Tablet Liv-52 was chosen as a standard drug while Unani formulation *kabdeen* as test drug. These drugs were given in both the groups for 60 days and the statistical analysis was carried out at appropriate intervals.

Here it is worth mentioning that our core of concentration was to see the clearance the Hepatitis-B surface antigen (HBsAg) from the blood in both the groups. Besides this, of course the amelioration in clinical features and biochemical abnormalities were also our focus of study. The diagnosis was made on the basis of clinical features and serological examination.

Ethical Clearance and Consent

The trial was carried out after the approval of departmental ethics committee and informed written consent.

Study Location

The study was conducted at outdoor and indoor sections of department of Moalijat, Ajmal Khan Tibbiya College Hospital, Aligarh Muslim University, Aligarh.

Study Size

The study included 20 cases of *Warm-e-Kabid Haad Vairoosi* (Acute Viral Hepatitis) Inclusion/Exclusion Criteria:

The patients in whome the presenting features was jaundice due to acute viral hepatitis B were included in the study. The patients suffering from surgical jaundice,

hypothyroidism, hyperthyroidism liver cirrhosis, and diabetes mellitus chronic renal failure, nephritic syndrome, use of estrogen containing oral contraceptives, chronic alcoholics, and having primary gout, were excluded from the study. Similarly smokers and those taking hypolipedemic drugs nicotinic acid statin and cortisone were excluded from this study.

Duration of study

Total duration of study was 60 days.

Treatment, Route and Dose

This clinical trial was concerned with comparison between Unani formulation 'Kabdeen 'and Ayurvedic formulation Liv-52 in the treatment of *Warm-e-Kabid Haad Vairoosi*. The patients were divided into two groups A (Test) and B (Control) comprising of 10 each. In Test group syrup *'Kabdeen'* two tea spoon full (10ml) 8 hourly and in Control group Tab Liv-52 two Tab 12 hourly was given orally for 60 days.

Parameters Studied

In the present study clinical / biochemical / serological parameters studied were jaundice, anorexia, nausea and vomiting, *artharalgia* and myalgia, headache, fever, itching, pain in right quadrant of abdomen, tender hepatomegally, dark urine, clay color stool, serum bilirubin, Transaminases, Alkaline phosphatase, Prothrombin time and HBsAg (Australian antigen).

Statistical Analysis

The values of the different clinical and biochemical parameters were compared witch each other and also with control group. All the results were statistically evaluated by applying paired t' test for the observation recorded before and after treatment.

Composition of syrup Kabdeen per 5ml is as follows:

S. No.	Constituents	Botanical name	Quantity (in mg)
1	Balchar (Root)	*Valeriana jatamasi* DC	80
2	Baranjasif (Leaves)	*Achillea mellifolium* Linn	160
3	Bekh-kasni (Root)	*Cichorium intybus* Linn	80
4	Chiraita sheeren (Whole Plant)	*Swertia chirata* Buch-Ham	80

S. No.	Constituents	Botanical name	Quantity (in mg)
5	Gul-e-ghafis (Flowers)	*Agrimonia eupatorium* Linn	80
6	Gul-e-nelofar (Flowers)	*Nymphaea alba* Linn	160
7	Gul-e-surkh (Flowers)	*Rosa damascena* Mill	80
8	Gul-e-tesu (Flowers)	*Butea frondosa* Koen.ex Roxb.	40
9	Kasondi (Leaves)	*Cassia occidentalis* Linn	80
10	Khulanjan (Root)	*Alpinia galanga*Willd.	40
11	Mako khushk (Leaves)	*Solanum nigrum* Linn	80
12	Nar mushk (Flowers)	*Mesua ferrea* Linn	80
13	Qand safaid (Crystals)	Cane sugar	4.5G.
14	Rewand Chini (Rhizome)	*Rheum emodi* Wall	80
15	Satar farsi (Whole plant)	*Zataria multiflora* Boiss	80
16	Shahtara (Leaves)	*Fumaria officinalis* Linn	160
17	Tukhm-e-bathooa (Seeds)	*Chenepodium album* Linn	40
18	Tukhm-e-kasni (Seeds)	*Cichorium intybus* Linn	160
19	Tukhm-e-kasoos (Seeds)	*Cuscuta reflexa* Roxb.	80
20	Tukhm-e-khyaren (Seeds)	*Cucumis sativus* Linn	160
21	Uood-e-Hindi (Stem)	*Aquilaria agalocha* Roxb.	80
22	Ushba maghribi (Root)	*Smilax aspera* Linn	80

***Composition of Liv-52*,** each Liv-52 Tablet contains

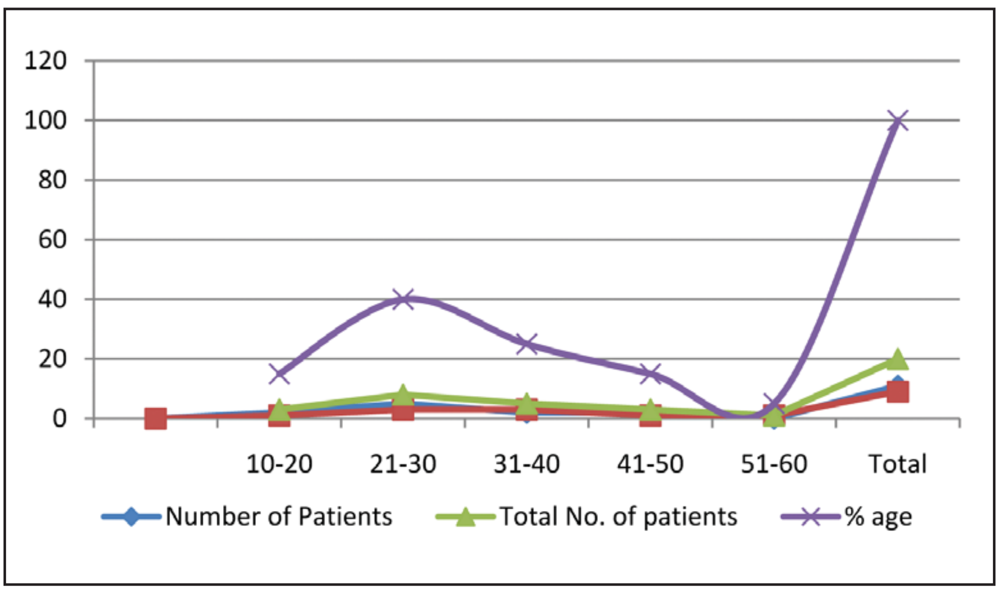
S. No.	Constituents	Botanical name	Quantity (in mg)
1	Himsra	*Capparis spinosa* Linn	65
2	Kasni	*Cichorium intybus* Linn	65
3	Mandura bhasma	*Ferric oxide calx*	33
4	Kakamachi	*Solanum nigrum* Linn	32
5	Arjuna	*Terminalia arjuna* W & A.	32
6	Kasamada	*Cassia occdentalis* Linn	16
7	Biranjasipha	*Achillea millefolium* Linn	16
8	Jhavuka	*Tamarix gallica* Linn	16

Discussion

All the patients in this study were between 10-60 years of age. However the maximum incidence was found to be between 20-30 years of age followed by 30-40 years of age. The male gender predominated the reason may be due to the shaving habits in barber's shop and untold extramarital relationship (Table 1).

Table 1: Showing Distribution of patients according to age and sex n =20

S. No.	Age group (in years)	Number of Patients		Total No. of patients	% age
		Male	Female		
1	10-20	2	1	3	15
2	21-30	5	3	8	40
3	31-40	2	3	5	25
4	41-50	2	1	3	15
5	51-60	0	1	1	5
	Total	11	9	20	100.0

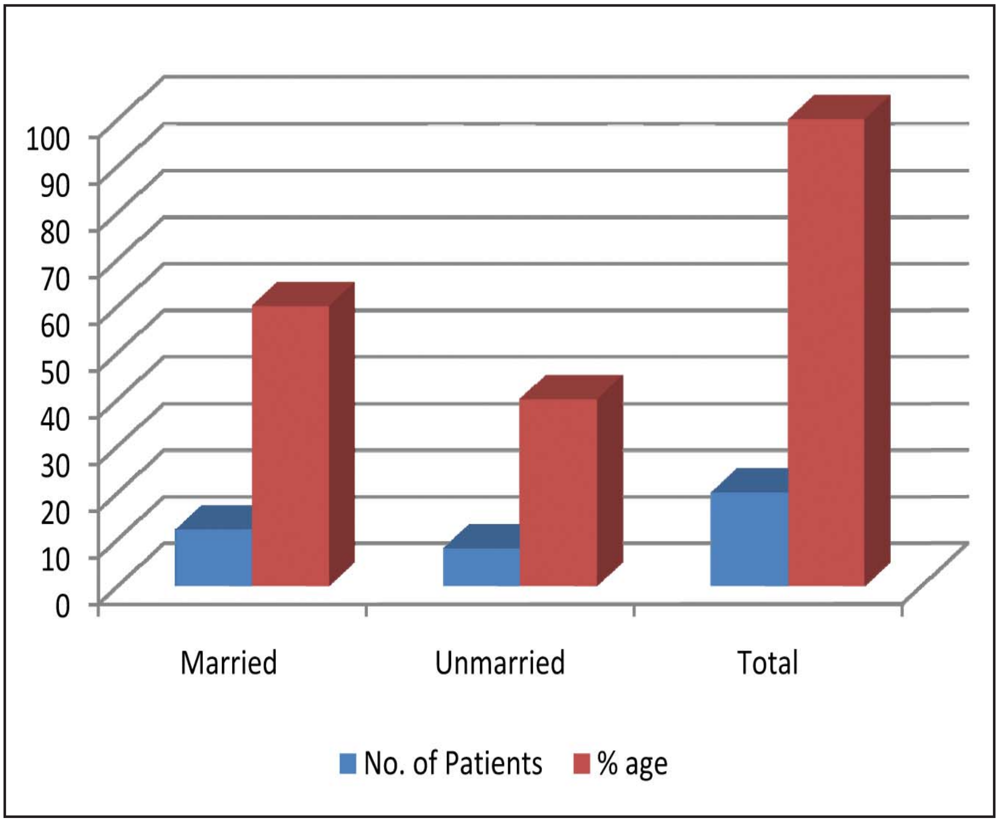


Graph 1: Showing distribution of patient according to age and sex

The incidence was definitely more in married that is 12 (60%) as compared to unmarried person that is 8 (40%) the most obvious reason seems to be through sexual transmission in married couples (Table 2).

Table 2: Showing distribution of patients according to marital status n = 20

S.No.	Marital Status	No. of Patients	% age
1	Married	12	60
2	Unmarried	08	40
	Total	20	100



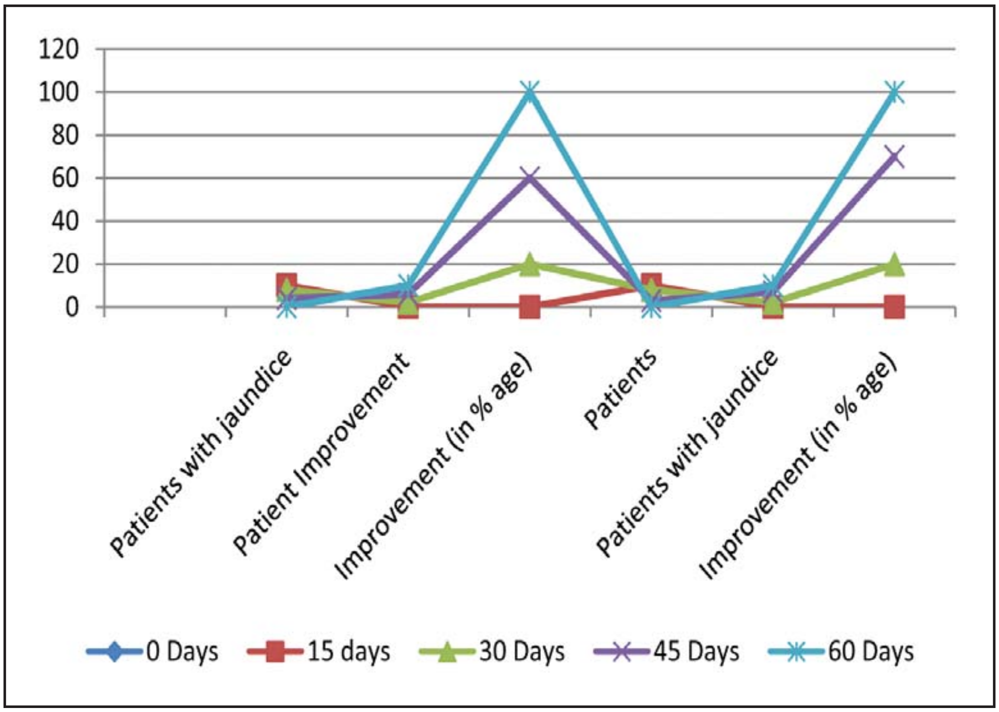
Graph 2: Showing distribution of patient according to marital status

In both the groups clinic jaundice was present in all the patients. Which disappeared completely in after completion of therapy that is 60 days In the test group the improvement may be due to the presence of *Kasni, Mako, Gul-e-Tesu, Revand Chini, Sumbuluteeb, Tukhm-e-Bathoua and Oh-e-Hindi* which are useful clearing the excess bile pigments from blood. (Chopra *et al.*, 1958; Ghazrooni,1311 H; Ibn-e-Baitar,1291H; Nadkarni, 1986).

The slightly better improvement in control group may be due to the additional action of *Kibar, Arjun and Mykala* which has diuretic anti-inflammatory and hepato tonic effect (Table 3).

Table 3: Showing effect of drugs on jaundice n = 20

Duration in days	Before Treatment 0 Days	15 Days	30 Days	45 Days	60 Days
Kabdeen	n = 10				
Patients with jaundice	10	10	08	04	00
Patient Improvement		00	02	06	10
Improvement (in % age)		00	20	60	100
Liv– 52	n = 10				
Patients	10	10	08	03	00
Patients with jaundice		00	02	07	10
Improvement (in % age)		00	20	70	100

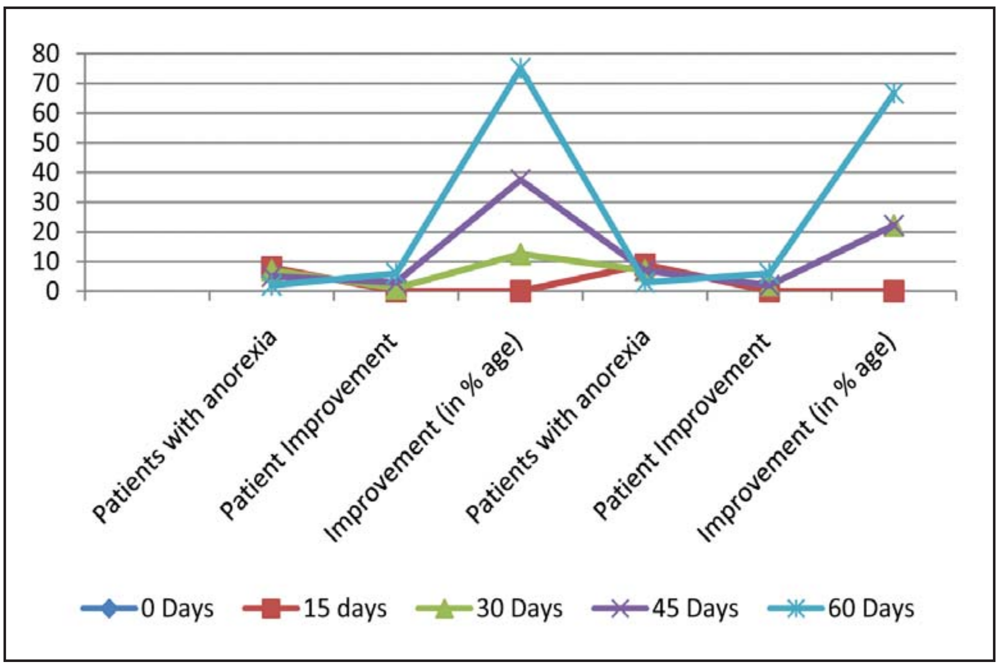


Graph 3: Showing effect of drugs on jaundice

As regard anorexia the improvement was seen in 75% of cases in test group and 66.66 in control group. *Kabdeen* has maximum beneficial effect as compared to control group. the improvement in test group may be due to the digestive and carminative effects of *branjasaf, kasni, mako, gul-e-tesu, sumbuluteeb, satarfarsi.* (Chopra *et al.*, 1958; Ghani,1921; Ibn-e-Baitar,1291H; Goswami,1984).

Table 4: Showing effect of drugs on Anorexia n = 20

Duration in days	Before Treatment 0 Days	15 Days	30 Days	45 Days	60 Days
Kabdeen	n = 8				
Patients with anorexia	8	08	07	05	02
Patient Improvement		00	01	03	06
Improvement (in % age)		0.0	12.5	37.5	75.0
Liv – 52	n = 9				
Patients with anorexia	9	09	07	07	03
Patient Improvement		00	02	02	06
Improvement (in % age)		0.0	22.22	22.22	66.66

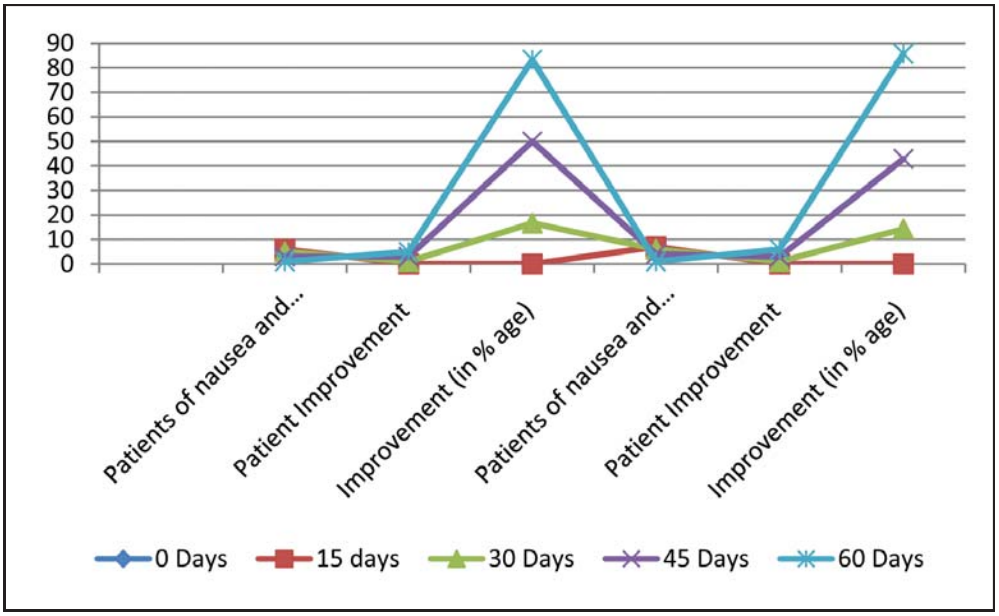


Graph 4: Showing effect of drugs on anorexia

As for as the nausea and vomiting are concerned, 83.33% in Test group while 85.71% of cases in Control group showed improvement. The improvement in Test group show almost similar pattern which may be due to the carminative and digestive effect of *Mako, Kasni, Gul-e-Surkh, Neelofar, Od-e-Hindi, Mayenkalan, Kibar* which are present in test formulaion. (Chopra *et al.*, 1958; Ghani, 1921; Ibn-e-Baitar,1291H; Dhar *et al.*,1968).

Table 5: Showing effect of drugs on Nausea and Vomiting n = 13

Duration in days	Before Treatment 0 Days	15 Days	30 Days	45 Days	60 Days
Kabdeen	n = 6				
Patients of nausea and vomiting	6	06	05	03	01
Patient Improvement		00	01	03	05
Improvement (in % age)		0.0	16.66	50	83.33
Liv-52	n = 7				
Patients of nausea and vomiting	7	07	06	04	01
Patient Improvement		00	01	03	06
Improvement (in % age)		0.0	14.28	42.85	85.71



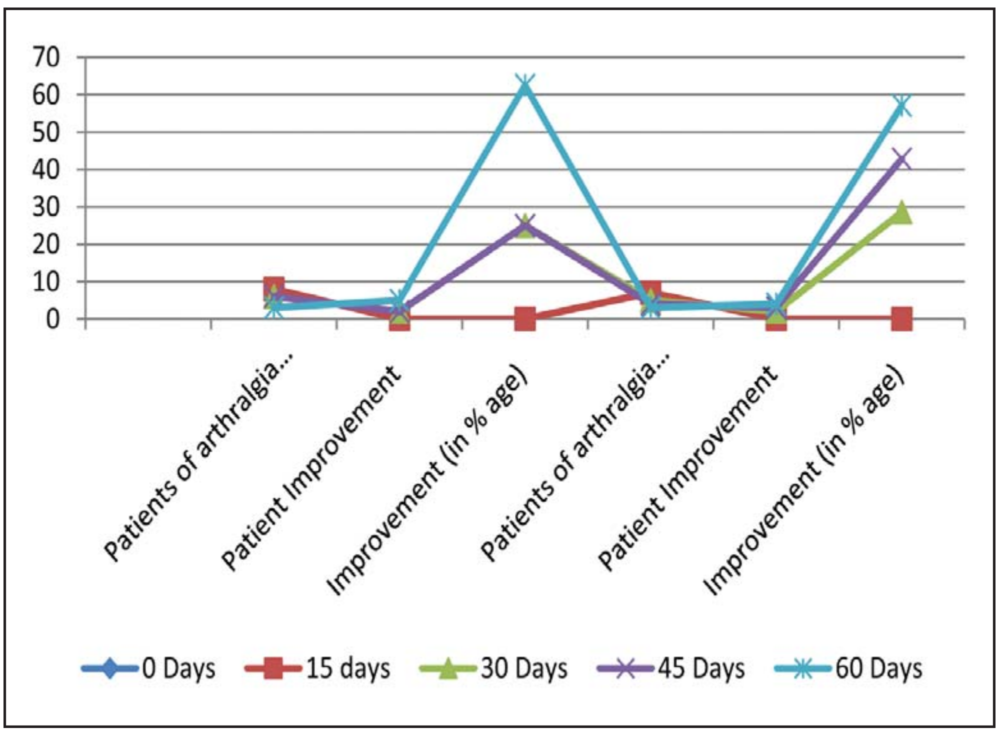
Graph 5: Showing effect of drugs on nausea and vomiting

Artharalgia and Myalgia are clinically important feature of Hepatitis-B as compared to other type of Viral Hepatitis. Those who received Kabdeen had maximum improvement while in Liv-52 only 4 patients showed improvement. This can be explained on the basis that analgesic activity of *Mako*, *Gul-e-Tesu*, *Revand cheeni*, *Gul-e-Neelofar*, *Uood-e-Hindi* and *Khulanjan* present in *Kabdeen.* (Chopra *et al.*, 1958; Ghani,1921; Ibn-e-Baitar,1291H; Goswami,1984).

Liv-52 as such contains one or two analgesic drugs. Apart from this autoimmune response and anti-serum sickness like effect of herbal drugs might be the cause of improvement of *Artharalgia* and Myalgia.

Table 6: Showing effect of drugs on *Artharalgia* and Myalgia n = 15

Duration in days	Before Treatment 0 Days	15 Days	30 Days	45 Days	60 Days
Kabdeen	n = 8				
Patients of *Artharalgia* and Myalgia	8	08	06	06	03
Patient Improvement		00	02	02	05
Improvement (in % age)		0.0	25	25	62.5
Liv-52	n = 7				
Patients of *Artharalgia* and Myalgia	7	07	05	04	03
Patient Improvement		00	02	03	04
Improvement (in % age)		0.0	28.57	42.85	57.14

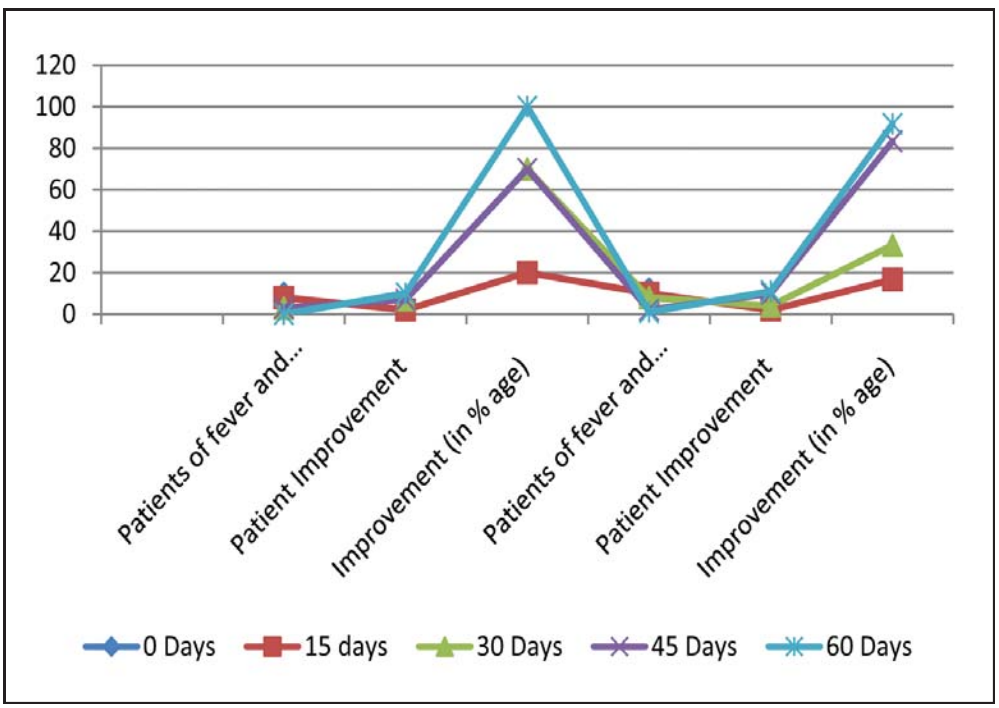


Graph 6: Showing effect of drugs on *artharalgia* and myalgia

The mechanism involved in ameliorating the fever and headache is probably the same as that in Artharalgia and Myalgia as agent that have analgesic property also have the antipyretic property.

Table 7: Showing effect of drugs on Fever and Headache n = 22

Duration in days	**Before Treatment 0 Days**	**15 Days**	**30 Days**	**45 Days**	**60 Days**
Kabdeen	n = 10				
Patients of fever and Headace	10	08	03	03	00
Patient Improvement		02	07	07	10
Improvement (in % age)		20	70	70	100
Liv-52	n = 12				
Patients of fever and Headace	12	10	08	02	01
Patient Improvement		02	04	10	11
Improvement (in % age)		16.66	33.33	83.33	91.67



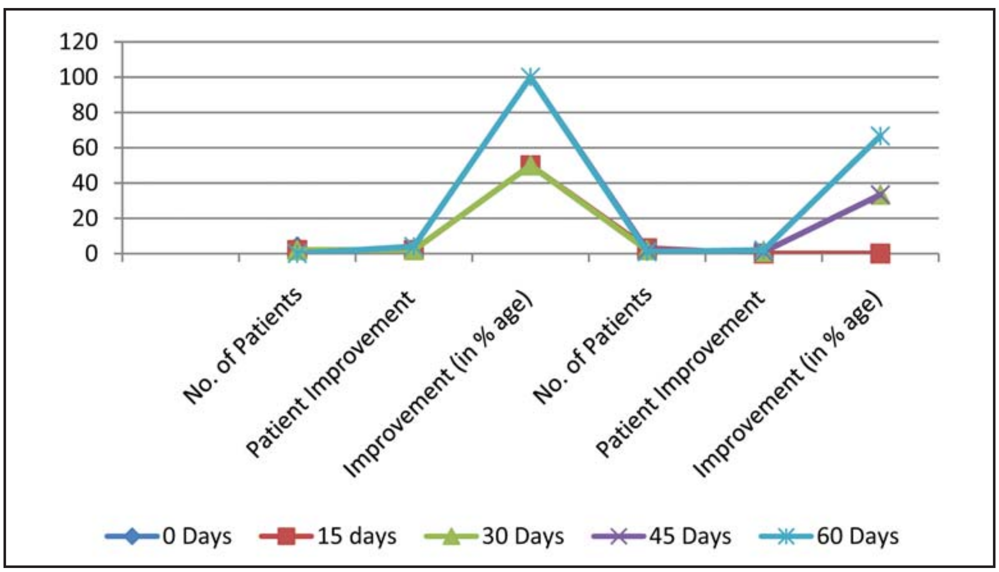
Graph 7: Showing effect of drugs on fever and headache

In the study, it was observed that the itching was the least common symptom amongst the entire symptom. In Test group after 45days of treatment itching disappeared whereas it persisted in one patient in Control group even after 60 days. This improvement may be due to *Mufatteh Sudud*, (Vasodilator), Cholerctic and anti-inflammatory action on *Kupfer* cells thereby facilitating flow of bile due to the presence of *Gul-e-Ghafis, Branjasaf, Mako, Kasni, Shahatra, Tukhm-e-Kasoos, Tukhm-e-Bathua, Khulanjan, Gul-e-Surkh.* (Ghani,1921; Ibn-e-Baitar,1291H;)

The reason of persistence of this symptom even after 60 days in Liv-52 group may be due to the absence of the property of *Tukhm-e-Kasoos, Satar Farsi, Branjasaf* which have *Mufattah-e- Sudud* action present in Kabdeen.

Table 8: Showing effect of drugs on Itching n =7

Duration in days	Before Treatment 0 Days	15 Days	30 Days	45 Days	60 Days
Kabdeen	n = 4				
No. of Patients	4	02	02	00	00
Patient Improvement		02	02	04	04
Improvement (in % age)		50	50	100	100
Liv-52	n = 3				
No. of Patients	3	03	02	02	01
Patient Improvement		00	01	01	02
Improvement (in % age)		0.0	33.33	33.33	66.67



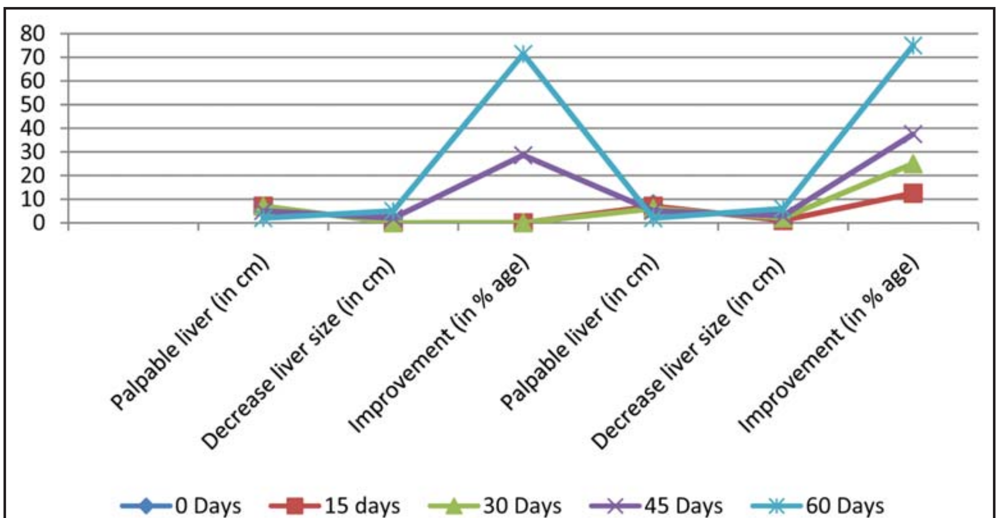
Graph 8: Showing effect of drugs on itching

In the present study 15 patients had this signs. The mean enlargement below the right sub-costal margin was 7 cm in Kabdeen, and 8cm in Liv-52 group. The enlargement in Kabdeen remained same up to 30 days whereas in Liv-52 and Lamivudin group, it decreased by 2 cm.

After 45 days there was a reduction of 2 cm in Kabdeen group and 3 cm in Liv-52 group. At the termination of the trial the liver size decreased by 5 cm in Kabdeen group and 6 cm in Liv-52 group. 75% improvement was seen in Liv-52 and 71.42% in Kabdeen group. The reasons for the decrease in size of liver almost probably the same as discussed in the earlier Table.

Table 9: Showing effect of drugs on tender hepatomegally in (cm)
Decrease Liver Size in Percentage n = 15

Duration in days	Before Treatment 0 Days	15 Days	30 Days	45 Days	60 Days
Kabdeen	n = 7				
Palpable liver (in cm)	7	07	07	05	02
Decrease liver size (in cm)		00	00	02	05
Improvement (in % age)		0.0	0.0	28.57	71.43
Liv-52	n = 8				
Palpable liver (in cm)	8	07	06	05	02
Decrease liver size (in cm)		01	02	03	06
Improvement (in % age)		12.5	25.0	37.5	75.0

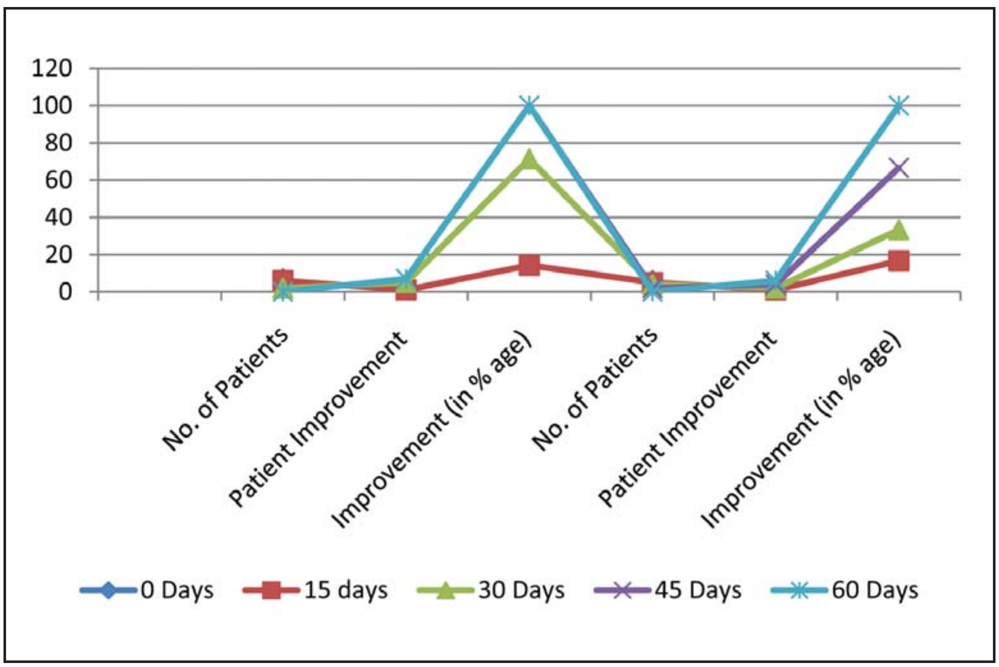


Graph 9: Showing effect of drugs on tender hepatomegaly in (cm)
(Decrease Liver Size in Percentage)

The urine color which was dark yellow due to the presence of urobilinogen before starting the trial reverted to its normal straw color after the completion of therapy in both Test and Control group which shows 100% improvement in Test group due its known diuretic and anti inflammatory activity of *Mako, Kasni, Khayarain* and *munzij* effect of *Mako* and *Baranjasaf.* (Ghani,1921; Ibn-e-Baitar,1291H;) Whereas in control group it took a longer time to achieve the same result because of lacking of drugs having diuretic and anti inflammatory activity.

Table 10: Showing effect of drugs on dark urine n = 13

Duration in days	Before Treatment 0 Days	15 Days	30 Days	45 Days	60 Days
Kabdeen	n = 7				
No. of Patients	7	06	02	00	00
Patient Improvement		01	05	07	07
Improvement (in % age)		14.28	71.42	100.0	100.0
Liv-52	n = 6				
No. of Patients	6	05	04	02	00
Patient Improvement		01	02	04	06
Improvement (in % age)		16.66	33.33	66.66	100.0



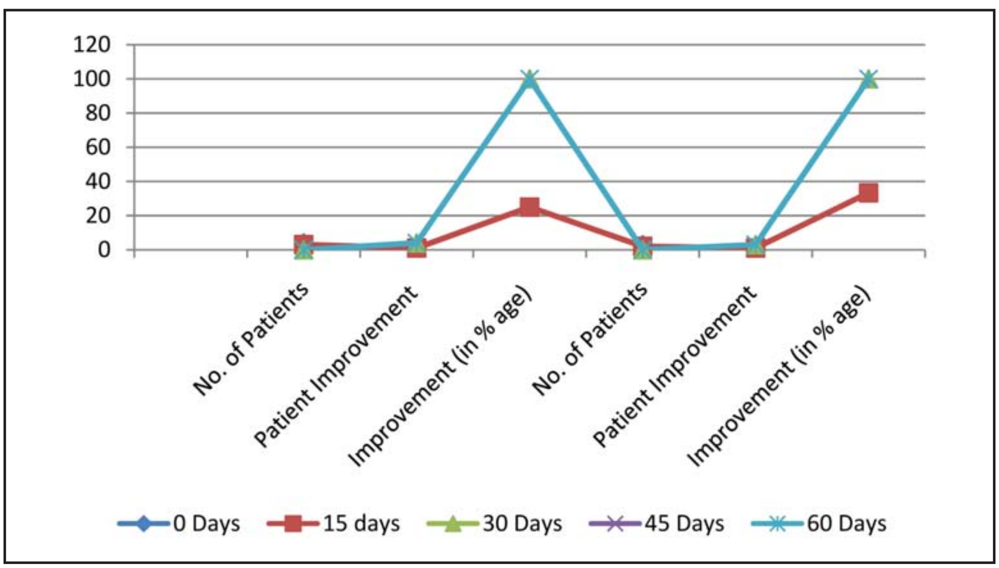
Graph 10: Showing effect of drugs on dark urine

The acute viral hepatitis passes through different stages one of which is the obstructive phase in the initial part of pre-clinical or clinical illness. This stage is due to the edema and obstruction in the flow of bile into the G.I.T which gives stool its characteristic clay color.

In Test group as well as in control group stool regained its normal color after 30 days of treatment. The regaining of normal stool color in test group is most probably due to the natural and uncomplicated course of illness or due to the anti inflammatory action of *Mako*, *Kasni*, *Branjasaf* and *Uood*-e-*Hindi* which reduces the edema of the intra hepatic bile canalicului. (Ghani,1921; Ibn-e-Baitar,1291H;) In Control group the similar pattern is observed. Probably same mechanism involved in control group also.

Table 11: Showing effect of drugs on clay color stool n = 7

Duration in days	Before Treatment 0 Days	15 Days	30 Days	45 Days	60 Days
Kabdeen	n = 4				
No. of Patients	4	03	00	00	00
Patient Improvement		01	04	04	04
Improvement (in % age)		25.0	100.0	100.0	100.0
Liv-52	n = 3				
No. of Patients	3	02	00	00	00
Patient Improvement		01	03	03	03
Improvement (in % age)		33.33	100.0	100.0	100.0



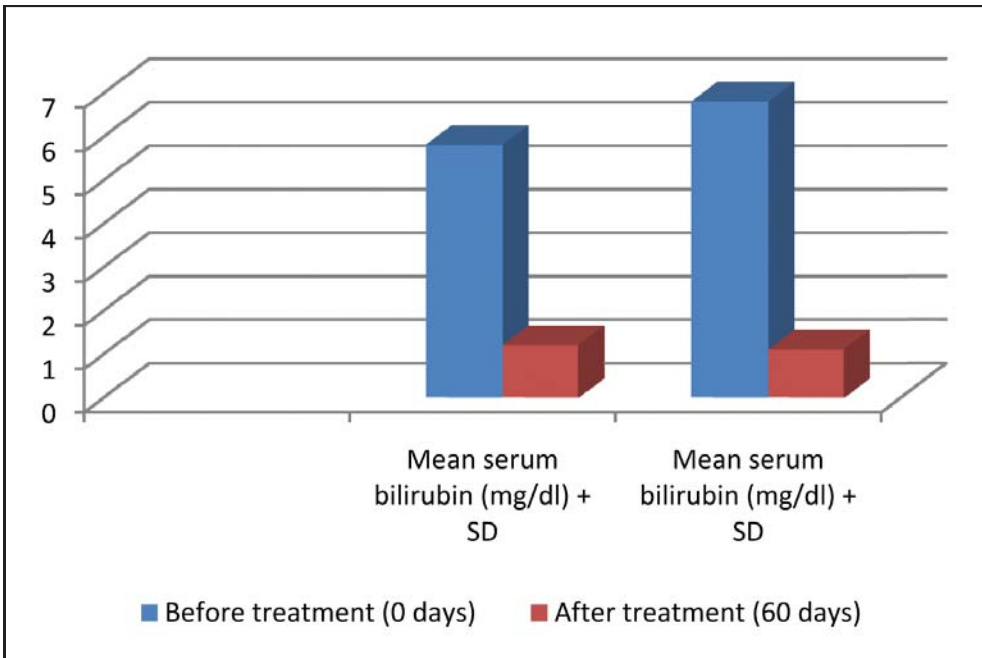
Graph 11: Showing Effect of drugs on clay colour stool

As it is evident from the above tables that the mean serum bilirubin before starting the treatment in both the groups was 5.8 mg %, 6.8 mg respectively which after completion of the therapy i.e. 60 days showed 79.32%, 83.82% improvement respectively.

The improvement in Kabdeen and Liv-52 groups may be due to the anti-inflammatory, cholangogue, cholekinetic, cholereteric and diuretic action of the drugs like *Mako, Kasni, Shahatra, Uood-e-hindi, Kiair, Myenkalan*, and *Arjun*. The marginal superiority in the Liv-52 group may be due to the presence of *Kiair, Myenkalan* and *Arjun.* (Sultana,2001; Chopra *et al*.,1958; Ghani,1921; Ghazrooni,1311H; Ibn-e-Baitar,1291 H; Goswami,1984).

Table 12: Showing effect of drugs on mean serum bilirubin

Parameters	Before treatment (0 days)	After treatment (60 days)
Kabdeen		
Mean serum bilirubin (mg/dl) $\pm$ SD	5.8 $\pm$ 1.69	1.2 $\pm$ 1.65
		N = 10, t = 3.0, p < 0.02
Liv-52		
Mean serum bilirubin (mg/dl) $\pm$ SD	6.8 $\pm$ 1.53	1.1 + 1.73
		N = 10, t = 5.3, p < 0.001

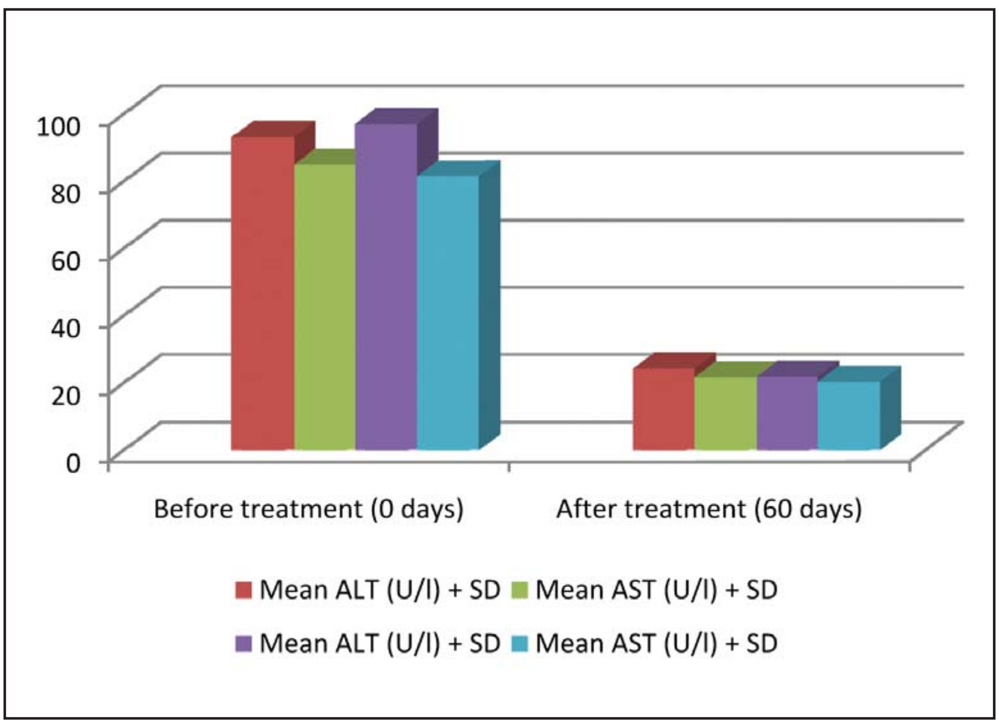


Graph 12: Showing the effect of drug on mean serum bilirubin

The transaminases activity reached within normal after 45 days in Test group as well in Control group. However, greater reduction was observed in Test group. The improvement may be due to the *Mako* and *Kasni* which have hepatotonic effects. Improvement was also observed in control group but at a slower pace.

Table 13: Showing effect of drugs on Transaminases

Parameters	Before treatment (0 days)	After treatment (60 days)
Kabdeen		
Mean ALT (U/l) ± SD	93.00 ± 1.47	24.00 ± 1.58
N = 10, t = 2.6, $p < 0.05$		
Mean AST (U/l) ± SD	84.75 ± 1.57	21.44 ± 1.49
N = 10, t = 2.8, $p < 0.002$		
Liv-52		
Mean ALT (U/l) ± SD	96.78 ± 1.86	21.65 ± 1.65
N = 10, t = 1.91, $p < 0.005$		
Mean AST (U/l) ± SD	81.25 ± 1.41	20.13 ± 1.88
N = 10, t = 2.80, $p < 0.001$		

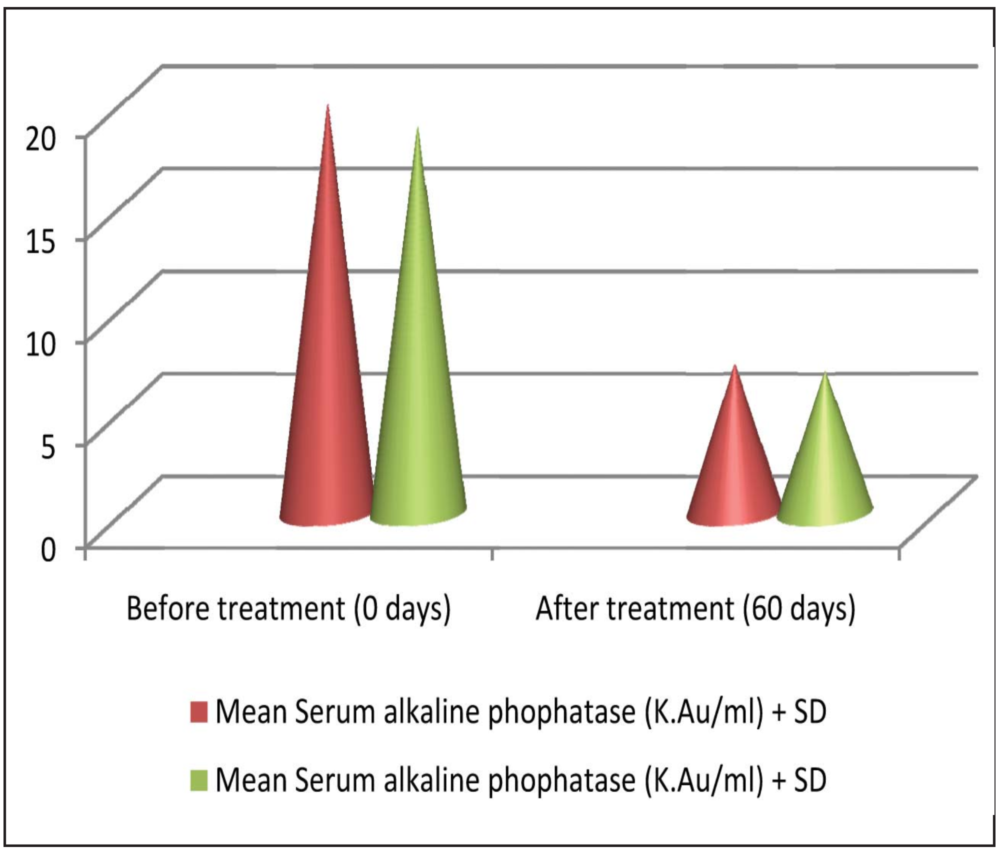


Graph 13: Showing effect of drugs on transaminases

The improvement in both groups may be attributed due to the presence of Unani and Ayurvedic medicine which have diuretic anti-inflammatory and hepatotonic actions thereby by decreasing the edema of kuffer cells facilitating the flow of excess serum alkaline phosphatase and bile.

Table 14: Showing effect of drugs on Alkaline Phosphatase

Parameters	Before treatment (0 days)	After treatment (60 days)
Kabdeen		
Mean Serum alkaline phophatase (K.Au/ml) ± SD	19.88 ± 1.44	7.2 ± 1.79
N = 10, t = 4.09, p < 0.001		
Liv-52		
Mean Serum alkaline phophatase (K.Au/ml) ± SD	18.76 ± 1.60	6.89 ± 1.49
N = 10, t = 3.3, p < 0.01		

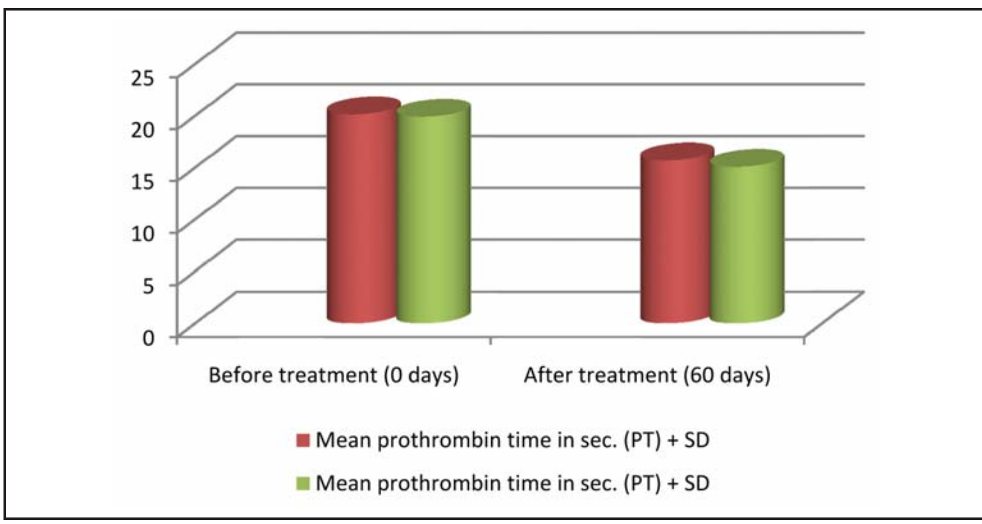


Graph 14: Showing effect of drugs on alkaline phosphatase

The synthesis of coagulation factors is one of the most important function of the liver which may be deranged partially or near completely in any form of viral hepatitis especially hepatitis B viral infection. It is very sensitive indicator of liver function. Its degree of prolongation is directly proportional to the extent of liver damage. While going through the above tables it will be observed that mean Prothrombin time not reached within the normal limits. However, there is also an improvement in both groups. These results prompts that there is every likelihood that if the duration of the study would have been longer Prothrombin time would also have reached within normal limits i.e. around 14 seconds. However the fall is uncourageous in the two groups which may be due to the presence of *Mako, Kasni, Branjasaf, Gul-e-Tesu, Cehraita* and *Arjun*. *Cheraita* which has androgroupholide alkaloid might have stimulated the coagulation mechanism besides hepatoprotective effects.

Table 15: Showing effect of drugs on Prothrombin Time

Parameters	Before treatment (0 days)	After treatment (60 days)
Kabdeen		
Mean prothrombin time in sec. (PT) ± SD	20.11 ± 1.88	15.66 ± 1.75
N = 10, t = 2.84, p < 0.02		
Liv-52		
Mean prothrombin time in sec. (PT) ± SD	19.88 ± 1.49	15.04 ± 1.68
N = 10, t = 3.24, p < 0.01		

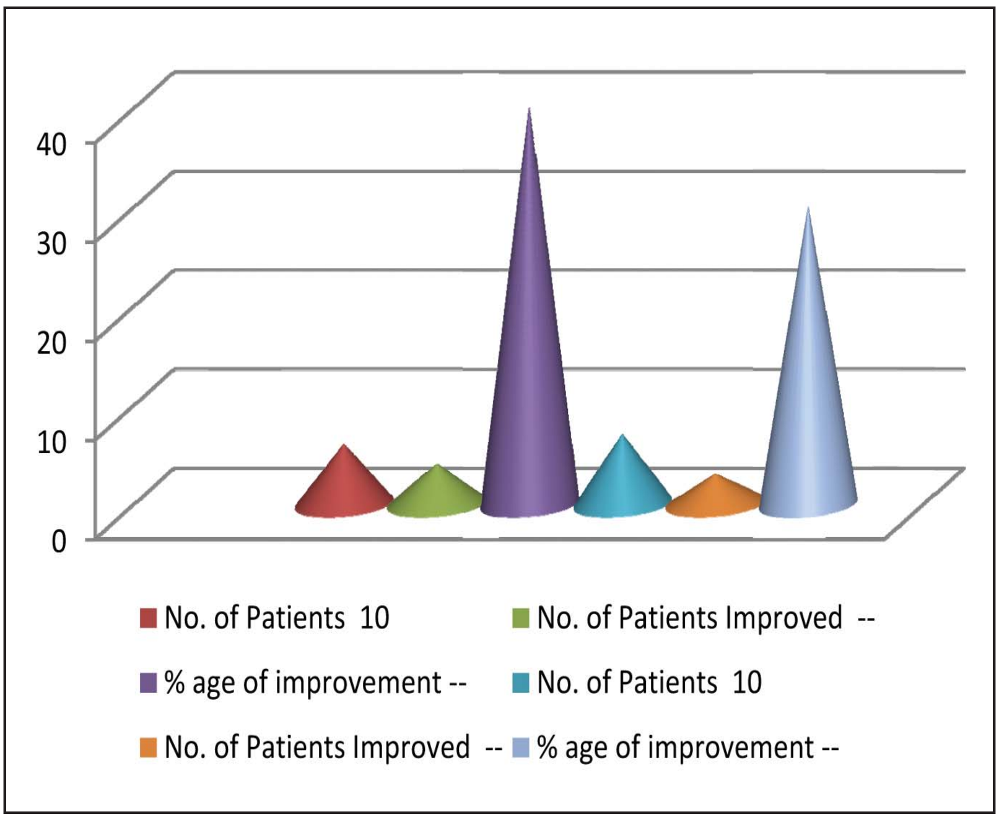


Graph 15: Showing effect of drugs on prothrombin time

As already mentioned only HBsAg (Australia Antigen) positive cases were included in this study. Besides noting overall improvement in sign, symptom and liver function test, our main aim and core of concentration was to know whether any of these drugs converts HBsAg +ve patients to HBsAg –ve status.

Table 16: Showing effect of Kabdeen on HBsAg (Australia Antigen)

Parameters	Before treatment (0 days)	After treatment (60 days)
Kabdeen n = 10		
No. of Patients	10	6 +ve
No. of Patients Improved	—	04
% age of improvement	—	40
Liv-52 n = 10		
No. of Patients	10	7 + ve
No. of Patients Improved	—	03
% age of improvement	—	30



Graph 16: Showing effect of Kabdeen on HBsAg (Australia Antigen)

From observations, it was noted that none of the cases became HBsAg –ve during 60 days treatment. Moreover, some encouraging results are definitely observed that is in the kabdeen group 6 patients remain positive while in liv-52 group 7 patients remained positive. It cannot be clearly deduced that whether this was a natural course of disease or it was because of drugs.

Statically kabdeen group produce the maximum negativity about HBsAg positivity. As for as mechanism of action is concerned it is very difficult to explain the clearance of HBsAg from the blood and it requires exhaustive immunological study. Most probably it may be due to the antiviral activity of one or the other ingredients in our Unani and Ayurvedic formulations.

Conclusion

On the basis of above results and discussion it can be concluded that both the trial drugs (kabdeen and Liv 52) are effective in alleviating the subjective symptoms in the patients of hepatitis B. These drugs also effective in normalizing the markers of liver function test incidentally this test drug is also effective against surface antigen HbsAg. Therefore, it can be concluded that the test drug can be safely used for the management of hepatitis B however long term study is required to elucidate the other pharmacological action and probable mechanism of action of test drug.

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Clinical Evaluation of Unani Drugs *Majoon Suranjan, Safoof Suranjan* and *Raughan Suranjan* in Waja-ul-Mafasil (Rheumatoid Arthritis) (A Preliminary Study)

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Abstract

Waja-ul-Mafasil is synonomously known as Rheumatoid Arthritis (RA). It is a chronic multi- system disease that affects joints, connective tissues, muscles, tendons and fibrous tissue. It occurs in about 3% of total population in the age group of 20-50 years. Females are three times more affected than males. RA manifests as a symmetric polyarthritis associated with swelling and pain in multiple joints, often initially occurring in the joints of hands and feet. The present study was conducted to evaluate the therapeutic efficacy of Unani pharmacopoeial formulations *MajoonSuranjan, SafoofSuranjan* and *RaughanSuranjan* in the management of *Waja-ul-Mafasil*. These classical Unani drugs significantly reduce joint pain ($p<0.05$), tenderness ($p<0.05$), morning stiffness ($p<0.05$), swelling ($p<0.05$), movement of restriction ($p<0.05$), and muscular weakness ($p<0.05$). The clinical and laboratory findings after treatment have shown that the trial drugs possessed efficacy in the treatment of Waja-ul-Mafasil.

This study demonstrates pronounced anti-arthritic effects indicating that these formulations would be potent drugs for arthritis patients.

Keywords: *Waja-ul-Mafasil, Majoon Suranjan, Safoof Suranjan* and *Raughan Suranjan*, Unani pharmacopoeial formulations.

Introduction

Rheumatoid arthritis is mentioned in classical Unani literature as *'Waja-ul-Mafasil'*. It is a condition of pain or an inflammation *(Waram)* which occurs in the joints of hands and feet, knee joints and ankle joints (Majoosi,1889; Ali, 1896; Jurjani, 1903). Shaikh Bu Ali Sina (Avicenna) (980-1036 AD) mentioned that *Waja-ul-Mafasil* is caused by phlegm *(Balgham),* blood *(Dam),* yellow bile *(Safra),* and black bile *(Sauda)* in a decreasing order of frequency respectively. *Waja-ul-Mafasil* is commonly caused by accumulation of viscid phlegm *(Balgham-e-Lazij)* in the joints due to weakness of the joints *(Zof-e- Mafasil)* (Majoosi, 1889; Khan, 1939). *Madda* (substance) causing *Waja-ul- Mafasil* enters into the joints and it neither absorbed nor expels from them due to lack of power of absorption *(Quwwat-e-Jazibah)* and power of expulsion *(Quwwat-e-Dafia)* in the joints, respectively and thus, it is retained in the joints, hence the nutrients reaching the joints are not properly utilized, instead they are converted into harmful products which induce inflammatory process. Thus, the *Waja-ul-Mafasil* is developed (Jurjani, 1903), when this *Madda* (substance) is retained in the joints for a long period, its viscosity

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(Ghilzat) and viscidity *(Luzoojat)* are increased and it becomes hard *(Tahajjur-e-Mafasil* or Osteoarthritis) and the condition is incurable (Majoosi, 1889). When the *Madda* (substance) which produces *Waja-ul-Mafasil* enters into the blood and permeates the entire body system, the non-articular manifestations are developed (Majoosi, 1889; Khan, 1939).The gist of all that follows: *Waja-ul-Mafasil*: when the joints suffer by pain the condition refers to *Waja-ul-Mafasil*.

It is mentioned that when the *akhlat* like *balgham* and *safra* come under *su-i-mizaj* (abnormal state), the condition becomes prone to *Waja-ul-mafasil*. It is a chronic multisystem disease characterized by a destructive and deforming polyarthritis, mainly affecting peripheral synovial joints, usually in a symmetrical pattern and a variety of non-articular manifestations In this condition joints lose their normal architecture resulted articulation disturbances, it is a condition of *sue-tarkeeb* and *tafarruq-wa-Ittissal* respectively (McGee *et al*., 1992; Kasper *et al*., 2005; Boon *et al*., 2006).

Materials and Methods

The study was designed as an open clinical trial to evaluate the efficacy of unani pharmacopoeial formulation on twenty nine patients of Waja-ul-Mofasil. The patients were treated for a period of eighty four (84) days with regular follow-ups after fourteen (14) days of interval at RRIUM, Patna. The patients suffering from Waja-ul-Mafasil were selected by adopting clinical criteria of Rheumatoid Arthritis Revised (1988) for diagnosis by the (ARA) American Rheumtism Association (Arnett *et al*., 1988). The inclusion criteria of the cases was done on the basis of pathological and biochemistry investigations (eg. RA, CRP), radiological changes and presence of joint pain, tenderness, morning stiffness, swelling, restriction of movement & muscular weakness. However, negative cases against RA and CRP were also included in the study on the basis of presence of above signs & symptoms.

Similar, the exclusion criteria was also maintained on the basis of serious disorders in renal, liver, cardiac and abnormality in any investigation (SGOT,SGPT, and ALP, SrCreatinine, B.Urea) done at the baseline. The cases, whose abnormality in investigations were found more than >2 ½ times from the normal value, drug induced, neurological, NES (Nerve Entrapment System), joint with permanent deformities, pregnant women and lactating mothers, were excluded from the study.

After written consent, the patients were enrolled for the treatment and laboratories investigations (HB%, TLC, DLC, ESR, LFT, KFT, Urine Routine, RAfactor, CRP and X-ray of affected joint) of the patients were also conducted. The investigations were repeated after treatment. The safety of the trial drugs was evaluated clinically by monitoring adverse effects which were carefully sought out on each follow-up.

The temperament (Mizaj) of the patients was assessed as per the parameter described in unani classical literature

All the signs and symptoms were assessed on each follow-up according to the flowing gradings.

Joint Pain: (1=Barely Perceptible; 2 =Mild: Can carry out daily activities with some trouble; 3= Moderate; cannot carry out daily activities easily, 4= Severe; bed ridden.)

Tenderness: (1=Patient says it is tender, 2=Patient says it is tender and winces, 3=Patient says it is tender, winces and pulls back, 4=patient does not allow palpation.)

Morning Stiffness: (1=Up to 15 minutes, 2=15 to 30 minutes, 3=30 to 45 minutes, 4=More than 45 minutes.)

Joint Swelling/ Effusion (0=No swelling/effusion, 1=Barely perceptible, 2=Mild, 3=Moderate, 4=Severe.)

Restriction of Movement: (1= Active range of motion (Partial voluntary movement), 2=Passive range of motion (Full movement, when the joint is moved by the examiner), 3= Passive range of motion (Partial movement, when the joint is moved by the examiner) Worst affected joint, 4= No movement at all.)

Muscular Weakness: (1=Strength against gravity and added resistance, 2=Strength only against gravity, not added resistance, 3=muscular contraction occurs, but not sufficient to overcome gravity, 4=Muscular contraction with little or no movement.)

Overall response was assessed on 90-100% - Complete Relief; 60-89% - Relief; 30-59% - Partial Relief and <30% - No Relief in the Improvement of sign & symptoms of disease. No concomitant treatment was allowed during the study. The observation results were statistically analyzed.

Objective of The Study

- To assess the clinical efficacy of unani pharmacopoiel formulation,*Majoon Suranjan, Safoof Suanjan & Raughan Suranjan* for symptomatic relief in patients of *Waja-ul-Mafasil* (RA).
- To assess the safety of unani pharmacopoiel formulation *Majoon Suranjan, Safoof Suanjan & Raughan Suranjan of Waja-ul-Mafasil* (RA).

Drug doses Schedule and Mode of Administration

1. Majoon-e-Suranjan

Dose: Trial drugs Majoon Suranjan (Semi-solid form-7gram) was given orally twice a day with water after meals.

Ingredients:

Name of the ingredients	Botanical Name	Qty
Suranjan Shireen	*Colchicum autmnale*	500 gm
Sana	*Cassia angustifolia* Vahl	250 gm
Zanjabeel	*Zingiber officinale* Rosc	100 gm
Zeera Siyah	*Carum carvi* L.	100 gm
Filfil draz	*Piper longum* L.	100 gm
Asaroon	*Asarum europaeum*	100 gm
Qand Safaid	*Saccharum officinarum* L.	3.5 kgs

2. Safoof-e-Suranjan

Dose: Safoof Suranjan (powder 6gram) was given orally twice a day with water after meals.

Ingredients:

Name of the ingredients	Botanical Name	Qty
Suranjan Shireen	*Colchicum autmnale*	25 gm
Buzidan	*Pyrethrum indicum* L.	25 gm
Post-e-Halela Zard)	*Terminalia chebula* Retz	25 gm
Maghz-e-Tukhme-e-Tarbooz	*Citrullus vulgaris* Schrad	25 gm
Maghz-e-badam	*Prunus amygalus* L.	25 gm
Maghz-e-Tukhm-e-Badranjboya	*Nepeta hindostana* (Roth) Haines	25 gm
Maghz-e-Tukhm-e-khaiyar-draz	*Cucumis sativus* L.	25 gm
Kishneez Khush	*Coriandrum sativus* Roxb.	25 gm
Tukhm-e-Khashkhash	*Papaver somniferum* Linn.	25 gm
Qandsafaid	*Saccharum officinarum* L.	225 gm

3. Roghan Suranjan

Dose: Raughan Suranjan (oil form) was also applied locally on the affected joints with gentle massage till it absorbed, twice a day in the morning and at bed time.

Ingredients:

Name of the ingredients	Botanical Name	Qty
Suranjantalkh	*Colchicum luteum* Baker	50 gm
Aab-e-Karafs	*Apium graveolens* L.	50 gm
Chiraita	*Swertia chirata* Buch. -Ham	25 gm
RoghanZaitoon	*Olea europaea* L.	150 gm

Laboratory Investigations

The following pathological, biochemical and radiological investigations were carried out before (baseline) & after therapy and six follow ups during 84days treatment.

Pathological Investigations

Blood for TLC (Total Leukocyte count), DLC (Differential Leucocyte Count), RBC (Red Blood Corpuscles), Hb%, ESR (Erythrocyte Sedimentation Rate), Urine R/E, were carried out. Besides these, for diagnosis of disease and assessment of response of therapy other pathological investigations CRP (C Reactive Protein) and RA (Rheumatoid Arthritis) factor for detection of Rheumatoid Arthritis were also carried out.

Biochemical Investigation

Biochemical investigations for example LFT (Liver Function Test), SGOT (Serum Glutamic OxaloaceticTransminase), SGPT (Serum Glutamic Pyruvic Transaminase), ALP (Serum Alkaline Phosphatase, Serum Bilirubin and KFT (Kidney Function Tests) Serum Urea and serum creatinine were carried out for safety of drugs.

Radiological Investigation

Radiological examination (X- ray of the affected joints) was carried out for assessment of the results.

Clinical Toxicity Evaluation

The toxicity of trial drugs was evaluated clinically by monitoring adverse effects which were carefully sought out at each follow up.

Assessment of Efficacy

The assessment of patients was done according to the subjective parameters

(joint pain, tenderness, morning stiffness, joint swelling, restriction of movement and muscular weakness) and the objective parameters (X-ray, ESR, CRP and RA factor).

As the subjective parameters differ in severity from patients to patients and an arbitrary grading, starting from 0 to 4 was improvised for appropriate assessment and statistical evaluation to evaluate the efficacy of the procedure. The clinical assessment of the patients was carried out after every 14 days and at the completion of the protocol the laboratory as well as radiological investigations were done before and after treatment. A comparative study was done against the basal clinical findings and the findings observed during first to sixth follow-ups. The difference, if any observed, was recorded in percentage and taken as the improvement caused by the respective treatment

Follow-up Evaluation

The clinical follow-up was carried out at regular intervals from 14^{th} day to 84^{th} day. The laboratory as well as radiological investigations was done before and after treatment.

Statistical Analysis

Statistical analysis was restricted to only those patients, who completed the full duration of study and followed the protocol. The statistical analysis were performed by Student's paired't' test. The result were expressed as Mean ± SD. Difference are considered significant at $p<0.05$.

Results and Discussion

The result regarding to the incidence of *Waja-al-Mafasil* (Arthritis) according to the demographic data and subjective parameters are as follows:-

Table 1: Classification according to stage of chronici

Chronicity (Month)	Clinical Parameters					
	Joint Pain	**Tender-ness**	**Morning stiffness**	**Joint swelling**	**Restriction of movement**	**Muscular weakness**
1-2 Months	01	08	16	12	15	13
3-4 Months	17	21	01	03	01	-
5-6 Months	06	-	04	09	-	-
>6 Above	05	-	08	02	01	-
TOTAL	**29**	**29**	**29**	**26**	**17**	**13**

As shown in the table no. 1, the 16, 15, 13 and 12 patients were having morning stiffness, restriction of movement, muscular weakness and joint swelling respectively in the 1-2 month of chronicity. The maximum 21 patients were having tenderness and 17 patients had joint pain of 3-4 month of chronicity. The maximum 09 patients had joint swelling from 5-6 month of chronicity. The maximum 08 patients were having morning stiffness and 05 patients joint pain from above month of chronicity. Out of 29 patients, 08 patients were having morning stiffness more than six months of chronicity of disease, 02 patients had joint swelling, and 01 patient had restriction of movements. No tenderness and muscular weakness was found in patients having 5-6 month and more than 6 month chronicity of the disease. However, no muscular weakness was found in 3-4 months chronicity of disease.

Table 2: Showing effected joints of patients

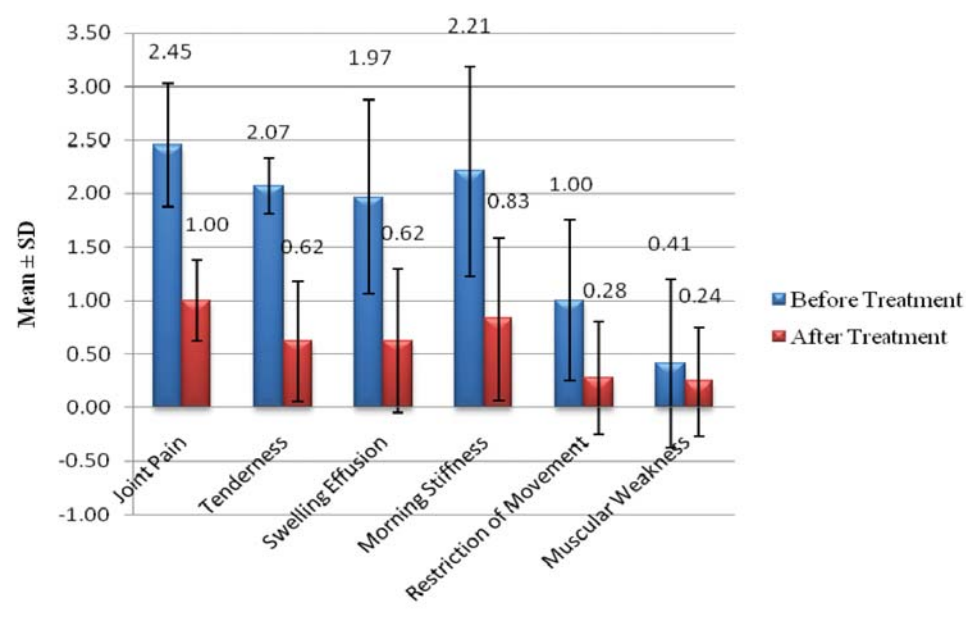
Joints Involved	Right	Left
Hip	10	–
Knee	23	21
Ankle	07	08
Shoulder	07	05
Elbow	05	03
Wrist	11	12
MCP Joints	09	06
PIP Joints	02	02
L S Joints	05	03
Cervical Joints	–	–

As shown the table no.2, maximum patients 23 & 21 were found left & right knee joints effected, next to it 11 and 12 patients were found left & right wrist joints and 1 & 2 patients were found left & right PIP joints. No patient was found to have cervical joint under the study.

As shown in the table No.3, the mean score of the joint pain before and after treatment was 2.45 and 01 which was 59% significantly. Similarly, the mean score of the tenderness before starting the treatment was 2.07 and however at the end of the treatment was 0.62. The improvement in tenderness at the end of the treatment was found 70.00%. The mean score of the swelling effusion before the treatment was measured 1.97. But at the end of treatment it was noticed was 0.62. The improvement in swelling effusion at the end of the treatment was found

Table 3: Effects of joints on subjective parameters

Parameters	Before Treatment	After Treatment	Improvement (%)
	Mean ± SD	Mean ± SD	
Joint Pain	2.45 ± 0.57	1 ± 0.38*	59.15
Tenderness	2.07 ± 0.26	0.62 ± 0.56*	70
Swelling Effusion	1.97 ± 0.91	0.62 ± 0.68*	68.42
Morning Stiffness	2.21 ± 0.98	0.83 ± 0.76*	62.5
Restriction of Movement	1 ± 0.76	0.28 ± 0.53*	72.41
Muscular Weakness	0.41 ± 0.78	0.24 ± 0.51*	41.67



68.42%. The related improvement in morning stiffness in before and after treatment was also shown. Before treatment it was 2.21 while after treatment it was 0.83. The improvement in this particular parameter was 62.50. Moreover, the mean score of the restriction of movement before and after treatment was 1 and 0.28 respectively. The improvement in restriction of movement statistically significant and it was 72.41%. And equally an improvement was also shown in muscular weakness and it was 41.67% from before and after treatment. The mean score of the muscular weakness before and after treatment was 0.41 and end of the treatment was 0.24.

Table 4: Showing response of drugs

Complete relief 90-100%	Relief 60-89%	Partial relief 30-59%	Not relieved < 30%	Total
00	20 (69%)	08 (28%)	01 (3%)	29 (100%)

Out of 29 cases, 20 (69%) patients found relief, 08 (28%) cases found partial relief and only 1 (3%) had no relief by examination of Unani classical drugs (Table No.4 and Figure No.4)

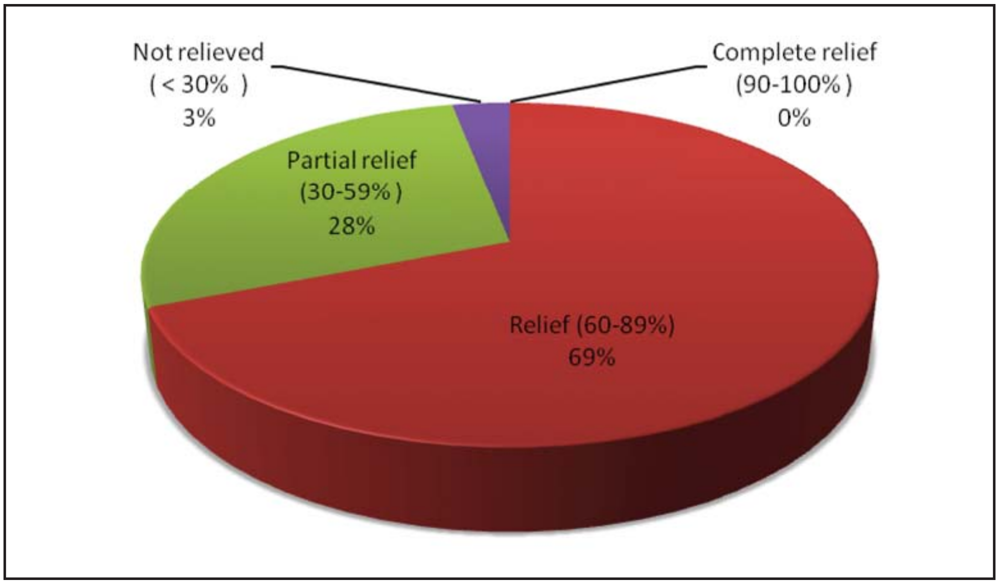
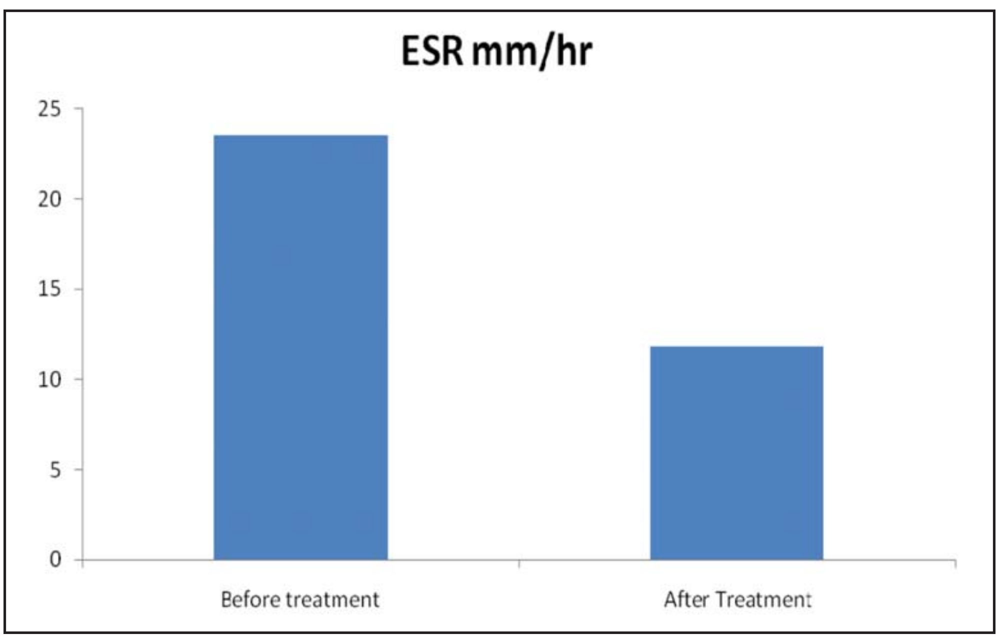


Figure 3: ESR mm/hr before and after treatment of Unani classical drugs



In the present study, the efficacy of Majoon-suranjan,Safoof- suranjan and Raughen- suranjan were evaluated over a period of 84 days on the basis of improvement in the subjective parameters. The improvement in joint pain, tenderness, morning stiffness, swelling, restriction of movement and muscular weakness was statistically significant and 59.15%, 70.00%, 68.42%, 62.50%, 72.41% and 41.67% respectively. Majoon suranjaan is reported as a antiarthritic property in Animal model (Singh *et al.*, 2011). Many single and compound unani drugs subjected to experimental and clinical studies have shown very promising results. For instance Suranjan Shireen (Colchicum autmnale) contain colchicine which is approved by USFDA for the treatment of Gout and familial medeterranean fever (Anonyms, 2014), Buzidan (Pyrethrum Indicum) was shown to produced significant analgesic and anti-inflammatory effect (Tajuddin, 1982). Maghz-e-Tukhme–e-Tarbooz *(Citrullus vulgaris*), Maghz-e-Tukhm-e-Badranjboya (Nepeta Hindostana) were shown to analgesic and antipyretic effects and compound formulations such as Safoof suranjan and Majoon suranjan (Ahmad,1997) have been reported to possess striking anti-arthritic activity and Raughen suranjan is a reputed and well-known drug in unani medicine and is used for locally application as anti-inflammatory and anti-arthritic agent. As main ingredients- Suranjan talkh (Colchicum luteum) and RoghanZaitoon (Olea Europea) were shown to anti-noceptive and anti-inflammatory effects (Nair, 2012; Fezai *et al.*, 2013)

Out of 29 cases, 18 cases were having RA factor positive and 24 cases had CRP positive and after the treatment of Unani drugs they had found negative. SGOT, SGPT, B. Urea and Serum creatinine were found in normal limit before and after treatment. There had no any change found in TLC, DLC, eosinophils, monocytes at baseline and after the treatment. Results suggest that the Unani classical drugs may be useful as potent drugs for the management of Waja-ul-Mafasil (RA). At the base line ESR increased, which was significantly reduced after treatment of Unani classical drug ($p<0.05$). Results are shown in the fig-3.

Conclusion

The classical Unani pharmacopoeial drugs, *MajoonSuranjan, SafoofSuranjan, RaughenSuranjan* in an unique combination of important Unani ingredients. Out of 29 cases, 20 (69%) were relief, 08 (28%) partially relief and 01 (3%) shows no response of drugs. It is also anti-inflammatory, antipyretic and analgesic actions reduced ESR, reduction of soft tissue and swelling, X-ray achieved and relief from clinical signs & symptoms. In view of the results achieved after studying 29 cases with the Unani Pharmacopoeial drugs. The study may be claimed as good result, safe remedy of arthritis. However, large controlled studies are required to reach at a final conclusion.

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Efficacy of 'Sharbat Bazoori Motadil' in The Management of Primary Dysmenorrhea *(Usre Tams Ibtidayee)*: A Clinical Observation

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Abstract

Usre tams Ibtidayee (primary dysmenorrhea) is painful menses in women with normal pelvic anatomy, usually beginning during adolescence and appearing within 6–12 months after the menarche. Objective of the present study was to evaluate the efficacy of 'Sharbat Bazoori Motadil' on the intensity of pain and others associated symptoms of *Usre tams Ibtidayee*. Patients suffering with regular and painful menstruation cycles were administered orally 20 ml of 'Sharbat Bazoori Motadil' with water, twice daily from 7th day before to the expected date of menstrual cycle for two consecutive cycles. It was found that after treatment the intensity of pain and associated symptoms; lumbago, lower abdomen pain, leg cramps and nausea were significantly ($p<0.05$) reduced by 50.7%, 88.4%, 66.2% and 73% respectively as compared to baseline. After treatment, reduction in the pain and others associated symptoms of *Usre tams Ibtidayee* was the main outcome measure. Highly significant improvements ($p< 0.001$, paired 't' test) in lower abdomen pain and nausea suggest that the results are really due to the therapy. In conclusion, Unani Paharmacopoeial formulation 'Sharbat Bazoori Motadil' was found effective in treatment of *Usre tams Ibtidayee* (Primary Dysmenorrhea) without any adverse effect on the human body.

Keywords: *Usre tams Ibtidayee*, Unani Medicine, Menstruation, Primary dysmenorrhea

Introduction

Usre-tams (dysmenorrhea) is defined as painful menstruation (Kantoori, 2007) and *Usre tams Ibtidayee* (primary dysmenorrhea) is painful menses in women with normal pelvic anatomy, usually beginning during adolescence (Avasarala *et al.*, 2008) and appearing within 6–12 months after the menarche. Primary Dysmenorrhea occurs almost invariably in ovulatory cycles. It is most common in women between the ages of 20 and 24 years, with most of the severe episodes occurring before 25 years of age (Dawood, 1987). About 88% of adolescents, experience their first painful menstruation within the 1- 2 years after menarche (Dutta, 1994). The cramps are most severe on the first or second day of menstruation. The pain is usually spasmodic in character and felt mainly in the lower abdomen, but it may radiate to the back and along the thighs (Abbaspour *et al.,* 2006). The cramp is commonly accompanied by one or more symptoms including nausea, vomiting, diarrhea, and lower backache etc. (Dutta, 1994; Khan, 1983; Gelani, 2007). Some 2–4 days before menstruation begins, prostaglandins proceed into the uterine muscle, where they build up quickly at menstrual onset

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and act as smooth muscle contractors that aid in the expulsion of the endometrium (Dawood, 1987).

According to Unani concept, the disease appears due to the dominance of phlegm and sauda. Menstrual blood become thick and its density is increased, which result less menstrual blood in quantity and the patients feel pain during the pre-menstrual cycle and during the Cycle (Azami, 1978). Swelling and accumulation of blood into the uterus are causes of this disease (Siddiqui, 2005). The famous Greek philosopher Hippocrates (460-377 BC) was of opinion that dysmenorrhea does not occur, when menstrual flow is regular and adequate in amount (Copeland *et al.,* 1993). According to Ibne sina, any obstruction in the flow of menstruation may cause usre tams; backache prior to menstruation is due to involvement of uterus (Kantoori, 2010). According to Zakarya Razi, causes of dard-rehm are warme rehm (inflammation of uterus), ihtebase tams (amenorrhea), sailan khoon (menorrhagia), sailan mani (leucorrhoea), displacement of uterus, cervical stenosis, uterine rupture, etc. (Al Razi, 2001). In usre tams menstrual blood is expel out in lesser amount (Khan, 1983) and women having scanty flow of menstruation usually suffer from pain (Kantoori, 2010). Mudir haiz drugs, which opens the blood vessels and makes blood lateef and hence induces the menstruation are recommended to relieve the pain, which occurs during menstruation. (Ibn Rushd, 1987; Khan, 2003)

Usre tams affects more than 50% of menstruating women in age group 18-25 years (Weisman *et al.*, 2009) and is a common cause of regular absenteeism of women in schools and work places resulting in loss of works hours and economic losses. (Padubidri *et al*., 2008; Moore *et al*., 1993). NSAIDS, hormonal pills and surgery intervention used in modern medicines produces more or less adverse effects in the human body therefore it is need of time to use Unani System of Medicine, which is safe, non-surgical, cost effective, having long lasting effect and almost no adverse effect in the human body.

The objective of this observational study was to evaluate the efficacy of 'Sharbat Bazoori Motadil', a Unani Pharmacopoeial formulation in the management of *Usre tams Ibtidayee*. (Anonymous, 1986)

Material and Methods

Study Drug: The study drug was 'Sharbat Bazoori Motadil' of which composition is given in Table-1 (Anonymous, 2006).

The present open observational study was carried out from April 2011 to March 2012 on 15 female patients of Usre tams in OPD of Regional Research Institute of Unani Medicine, Patna.

Table 1: Formulation of Sharbat bazoori Motadil of 10 ml preparation (Anonymous, 2006)

Sr.No.	Ingredients	Scientific names	Quantity
1	Bekh-e-Kasni	Cichorium intybus	1.818 gm
2	Bekh-e-Badiyan	Foeniculum vulgare	909 mg
3	Tukhm-e-Kasni	Cichorium intybus	909 mg
4	Tukhm-e-Khiyar	Cucumis sativus	909 mg
5	Tukhm-e-Khiyarza	Cucumis sativus	909 mg
6	Tukhm-e-Kharbuza	Cucumis melo	909 mg
7	Khar-e-Khasak-Khurd	Tribulus terrestris	909 mg
8	Qand Safaid	Saccharum officinarum	9.09 mg

Patients Selection:

The patients presenting signs and symptoms of *Usre tams* (Dysmenorrhea) i.e. lumbago, lower abdomen pain, leg cramps, nausea, vomiting, breast tenderness and diarrhea were subjected to inclusion and exclusion criteria before to their selection for this study.

Inclusion Criteria:

- Patients of *Usre tams* (Dysmenorrhea) between 18 - 30 years of age.
- Patients with regular menstrual cycle (28 $\pm$ 7 days).
- Patients with at least 4 painful menstrual cycles during the previous 6 months.

Exclusion Criteria:

- Patients with irregular menstruation cycles.
- Patients with underlying pelvic pathology
- Lactating or pregnant women.
- Diabetes patients
- Patients receiving oral contraceptives within 6 months prior to study entry.
- Patients receiving concomitant medications including antipsychotics, antidepressants, sedative hypnotics, antispasmodics, and corticosteroids.
- congenital malformations of genital tract
- Patients with abnormal uterine bleeding.

Diagnostic Criteria:

Diagnosis of each case was made with the help of history in respect of selected patients i.e. previous similar episode, physical and systemic examinations.

Treatment of Patients:

All patients of primary dysmenorrhea, selected as per the inclusion/exclusion criteria were diagnosed by giving them 20 ml of 'Sharbat Bazoori Motadil 'with water, twice daily before to 7th day of the expected date of menstrual cycle. The treatments were carried out for two consecutive menstrual cycles and two follow up on or after 15 days were taken in each month.

Safety Assessment:

The safety was assessed by monitoring adverse events volunteered by the patients or observed during the course of the study. No adverse effect of the 'Sharbat Bazoori Motadil' was either reported by the patients or was observed in OPD during the course of this study.

Statistical Analysis:

All the data at the baseline and after the treatment were statistically analyzed by applying paired 't' test. $p<0.05$ has been considered as statistically significant and $p < 0.001$ has been considered as statistically highly significant.

Observations

During the course of the study, patients were divided into three age groups viz. 18-20 years, 21-25 years and 26-30 years. It was observed that 7 cases (46.67 %), 5 cases (33.33 %) and 3 cases (20 %) belonged to age groups 18-20 years, 21-25 years and 26-30 years respectively. Among the 15 patients; 11 patients (73.33 %) were unmarried and 4 patients (26.67 %) were married**.** High incidence of disease was among the low age group 18-20 years i.e. 46.67% followed by the young women (33.33 %) of age group 21-25 years. This has been summarized in Table-2.

Patients were divided into four categories according to their occupations i.e. student, house-wives, teachers and private-service. The number of cases falling in each category was 6 (40%), 5 (33.33 %), 3 (20 %) and 1 (6.67 %) respectively. This has been mentioned in Table-3.

Patients were divided into 3 groups in accordance with their socio-economic status. Out of 15 patients enrolled in the study, 4 (26.67 %), 9 (60 %) and 2 (13.33 %) patients were belonged to Low Income Group (LIG), Middle Income Group (MIG) and Upper Income Group (UIG) respectively. This is mentioned in Table-3.

Table 2: Distribution of patients according to Age and Marital status.

Total No. of cases- 15

Age group (in years)	Married		Unmarried		Total	
	No.	% age	No.	% age	No.	% age
18-20	1	6.67	6	40	7	46.67
21-25	2	13.33	3	20	5	33.33
26-30	1	6.67	2	13.33	3	20
Total	4	26.67	11	73.33	15	100

Table 3: Distribution of patients according to their occupation and social status.

Total No. of cases- 15

Occupation	Social Status							
			Lower Income Group (LIG)		Middle Income Group (MIG)		Upper Income Group (UIG)	
	No.	% age	No.	% age	No.	% age	No.	% age
Student	6	40	2	13.33	3	20	1	6.67
House-wife	5	33.33	2	13.33	2	13.33	1	6.67
Teacher	3	20			3	20		
Private service	1	6.67			1	6.67		
Total	15	100	4	26.67	9	60	2	13.33

It was observed that 12 (80 %) patients were non-vegetarian and 3 (20 %) patients were vegetarian. High incidence of disease among the persons having non-vegetarian habits shows that they are more prone to dysmenorrhea. This has been shown in Table-4.

It was observed that life style of 11 (73.33%) patients were active and 4 (26.67%) were sedentary. High incidences of the disease were among the women with active life style. Thus young women, whose life-style are more active are more prone to this disease. This has been shown in Table-5.

Observation on reduction in intensity of the menstrual pain accompanied with one or more symptoms of Usre tams Ibtidayee like; lumbago, leg cramps, lower abdomen pain, nausea, vomiting, breast tenderness and diarrhea at the baseline and after the treatment with the Sharbat bazoori Motadil, have been depicted in Table-6 and Graph-1. Mean $\pm$ SEM scores of lumbago, leg cramps, lower abdomen

Table 4: Distribution of patients according to Dietary Habits.

Total No. of cases- 15

Dietary Habits	No. of Patients	Percentages (%)
Vegetarian	3	20.0
Non-vegetarian	12	80.0
Total	15	100.0

Table 5: Distribution of patients according to their life styles.

Total No. of cases- 15

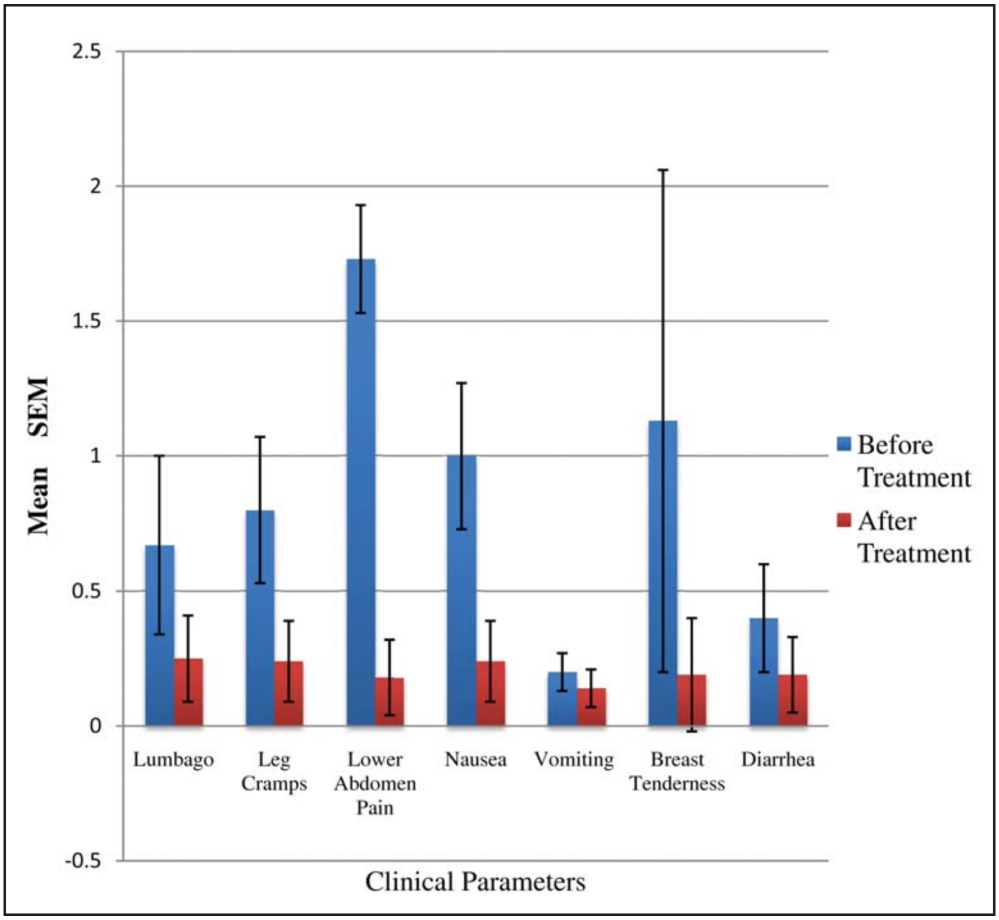
Life style	No. of Patients	Percentage
Sedentary	4	26.67
Active	11	73.33
Total	15	100

Table 6: Effect of 'Sharbat Bazoori Motadil' on clinical parameters of *Usre tams Ibtidayee* (primary dysmenorrhea)

Clinical Features	Mean ± SEM (Base line)	Mean ± SEM (After Treatment)	Percent Reduction	't'value	df	p value	Remarks
Lumbago	0.67 ± 0.25	0.33 ± 0.16	50.7	2.6458	14	<0.05	Significant
Leg Cramps	0.80 ± 0.24	0.27 ± 0.15	66.2	4.00	14	<0.05	Significant
Lower Abdomen Pain	1.73 ± 0.18	0.2 ± 0.14	88.4	11.5	14	<0.001	H.S.
Nausea	1.0 ± 0.24	0.27 ± 0.15	73.0	4.7845	14	<0.001	H.S.
Vomiting	0.20 ± 0.14	0.07 ± 0.07	65	1.4676	14	>0.05	N.S.
Breast Tenderness	1.13 ± 0.19	0.93 ± 0.21	17.6	1.8708	14	>0.05	N.S.
Diarrhea	0.4 ± 0.19	0.2 ± 0.14	50.0	1.8708	14	<0.05	N.S.

Statistical analysis by paired 't' test, n = 15, p<0.05 (Significant value), p<0.001 (Highly Significant value)

pain and nausea at the baseline i.e. 0.67 ± 0.25, 0.80 ± 0.24, 1.73 ± 0.18 and 1.0 ± 0.24 respectively, were found significantly reduced to 0.33 ± 0.16, 0.27 ± 0.15, 0.2 ±0.14 and 0.27 ± 0.15 respectively after the treatment. Scores of the clinical parameters before and after the treatment have been statically analyzed by



Graph 1: Effect of Sharbat Bazoori Motadil on Usre tams Ibtidayee (primary dysmenorrhea)

applying paired 't' tests. After the treatment, statically high significant decrease in the scores ($p<0.001$) were found in lower abdomen pain and nausea as compared to baseline.

Results and Discussion

In the present study, it was found that incidence of disease in women of age group 18-20 years and 21-25 years were 46.67% and 33.33% respectively i.e. (46.67% + 33.33%) 80% women in age between 18 to 25 years. This finding is in agreement with the study of Weisman A M that more than 50% of menstruating women are in age group 18-25 years (Weisman et al., 2009). It is also in consonance with the earlier observations that most of the severe episodes occur before 25 years of age (Dawood, 1987).

The prevalence of Usre tams Ibtidayee (primary dysmenorrhea) was found decreasing with increasing of age, indicating that (primary dysmenorrhea) Usre

tams Ibtidayee peaks in late adolescence and the early of 20 years and the incidence falls with increasing age.

Our results demonstrated that the prevalence of primary dysmenorrhea is dependent on family income. However, some researchers have indicated that economic status is not consistently associated with (primary dysmenorrheal) *Usre tams Ibtidayee* suggesting that further research is necessary by taking large sample to clarify this factor.

Highest incidence of disease was found in students (40%). Due to carrier building, students generally get married after twentieth and there is also greater awareness of the disease among the students. The incidence of menstrual pain is greatest in women in the late twenties and then declines with the age. This finding is in consonance with the described facts that primary dysmenorrhea is most common in women between the ages of 20 and 24 years, with most of the severe episodes occurring before 25 years of age (Dawood, 1987).

High incidence of the disease i.e. 73.33 % was found in women with active life style as compared to 26.67 % with sedentary life style. The women working in the offices in our present set up are under great social and cultural pressure and the high level of the stress is most significant predictors of the premenstrual symptoms and *Usre tams Ibtidayee*.

It was observed that high incidence i.e. 80 % cases were belonged to women having Non-Vegetarian habits. A low-fat, vegetarian diet reduces *Usre tams Ibtidayee* (Primary Dysmenorrhea) and premenstrual symptoms in women by its effect on serum sex-hormone binding globulin and estrogen activity. Ovulatory disturbances are also less frequent among vegetarians.

At the end of the study, the severity of lumbago, leg-cramp, lower abdomen pain and nausea were found significantly reduced by 50.7%, 66.2%, 88.4% and 73.0% respectively as compared to baseline. Decrease in symptoms like vomiting, breast tenderness and diarrhea were not found statically significant. After treatment, decrease in Mean $\pm$ SEM scores of the severity of lower abdomen pain and nausea were found highly significant ($p<0.001$) as compared to baseline, when the scores at baseline and after the treatment were statically compared by applying paired 't' test. Reduction in Mean $\pm$ SEM scores of the lumbago and leg cramp were found significant ($p<0.05$). The lumbago, leg-cramp, lower abdomen pain and nausea, which were the main causes of suffering of patients before and during the menstruation, were markedly reduced after the treatment. Reduction in intensity of lower abdomen pain, leg cramp, lumbago and nausea suggest that that relief to the patients suffering with *Usre tams Ibtidayee* (Primary Dysmenorrhea) was really due to the therapy but was not just a chance. Ingredients of compound drugs Sharbat bazoori Motadil has anti-Inflammatory, analgesic and mudir haiz

effect which opens the blood vessels and makes blood lateef and hence induces menstruation, ultimately it relieves the pain.

Conclusion

On the basis of above observations, it can be concluded that Unani Pharmacopoeial formulation 'Sharbat bazoori Motadil' is effective in management of *Usre tams Ibtidayee* (Primary Dysmenorrhea). The drug is cheaper, easily available and well tolerated by the patients without having any side effect. It can be propagated to use Sharbat bazoori Motadil for treatment of *Usre tams Ibtidayee* (Primary Dysmenorhea).

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Ethnopharmacological Studies Among the Tribal Communities of Udayagiri Forest Division of Nellore District, Andhra Pradesh, India

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Abstract

The present paper highlights the ethnopharmacological importance of the flora of Udayagiri forest division of Nellore district in the state of Andhra Pradesh. Tribal people inhabited in this area have good knowledge on medicinal potential of the available plant resources. They utilize different parts like roots, tubers, stem bark, leaves, flowers, fruits and seeds. All these parts are employed in the form of paste, powder, juice, decoction, infusion etc. The remedies are also used in crude form, with other additives like 'ghee', 'sesame oil' and 'goat milk', to get relief from different ailments.

The paper discloses hidden knowledge of tribal people on 41 species of plants belonging to 31 families to relieve the ailments like arthritis, Jaundice, rheumatism, burning micturation, fevers, worm infestation, menstrual problems, cold, cough, indigestion, diarrhoea, constipation, diabetes, asthma, stomach-ache, insomnia, piles,, sexual problems, bone fractures, skin diseases, ulcers, cuts, wounds etc.

Keywords: Ethnopharmacological studies, Tribal knowledge, Udayagiri forest division, Andhra Pradesh.

Introduction

History of herbal therapy is age-old in India. Since the ancient times, man has remained dependent on plants as a source of remedies. Traditional systems like Unani, Ayurveda and Siddha acquired their existence in India, due to the presence of rich biological diversity. India is a tropical country and home of extraordinary variety of climatic regions, ranging from tropical in the south to temperate and alpine in the Himalayan north, where elevated regions receive sustained winter snowfall. The climatic conditions are strongly influenced by the Himalayas and the Thar Desert (Joy, 2001; Chatterjee, 1953). Around 70% medicinal plants are found in tropical areas consisting of various forest types spread across the Western and Eastern Ghats; the Vindhyas, Chota Nagpur plateau and Aravalis. With these means India is a treasure of rich biological diversity and the majority of the medicinal plants get their home in tropical forests. Most of the tropical forests are located in Southern part of India. The state of Andhra Pradesh located in southern part of India; is a rich source of tropical forests with diverse flora. The Nellore district of the state has richest flora. Besides, it has centuries old experienced practices of herbal therapies as compared to other districts of the state (Pratap *et al*., 2010). In view of this rationale, an ethnopharmacological survey of the study area was conducted during 2011.

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The Study Area

The climate of Nellore district is tropical, maximum temperature of this area ranges from 36^0C to 46^0C during summer and the minimum from 23^0C to 25^0C during the winter. April and May are the hottest months. The coldest days are experienced during the months of December to February. This area receives seasonal rain fall up to 1000 mm, 60% of rainfall is received by the North- East monsoon which starts from October and continues till December.

The total geographical area of the district is 85,600 hectares. Out of this up to 40% area is used for agriculture and house hold purposes. The 21% is forest area and remaining is waste lands. The forest is tropical dry deciduous type. The medicinal importance of the flora of this district has been explored earlier Kirtikar and Basu, 1933; Sudarsanam and Rao, 1994; Pullaiah and Chennaiah, 1997; 2007; Bhakshu and Raju, 2007; Suryanarayana and Sreenivas, 2002 and Vedavathy and Mrudula, 1996).

The forests of this area are comprised of vast flora of medicinal plants like *Aegle marmelos* (L.) Corr.*, Cochlospermum religiosum* (L.) Alston.*, Vitex negundo* L.*, Vitex altissima* L.F.*, Alangium salviifolium* (L. F.) Wang. etc. Endemic plants of this area are *Pterocarpus santalinus* L.F.*, Shorea thumburgaia* Roxb.*, Terminalia pallida* Brandis. This area is inhabited by several tribal groups like the Chenchus, Yerukulas, and Yanadis or Irulas. They have been living in thick forest zones from immemorial days having no source to get modern medicine for their health care. The main source for their healthcare is forest herbs. They have good therapeutically valuable information for different ailments (Balaji Rao *et al.,* 1995 and Vijaykumar and Pullaiah, 1998.).

Materials and Methods

Ethnopharmacological data was collected through interaction with traditional healers, tribal doctors and elder people during survey trip conducted in 2011. During the interviews information on local name (s) of the plants, useful plant parts, method of preparation and dosage were recorded. The voucher specimens for each species was collected in quadruplicates, which were carefully tagged with field numbers after making a critical observation on the habit, habitat, colour and odour of flowers, phyto-association, occurrence and other relevant ecological features, which cannot be discerned from dried herbarium specimens (Pratap *et al.,* 2009, 2010; Hemadri 1984; Gupta *et al.,* 2003, 2005, 2007, 2009; Reddy *et al.,* 1989; Sudhakar and Rao 1985; Reddy *et al.,* 1989; Suryanarayana 1996 and Vedavathy and Mrudula, 1995). Medicinal uses and identification of plants specimens were authenticated with the help of published literature (Kirtikar and Basu, 1935; Crellin, 1967; Ramarao *et al.,* 1987; Ellis 1987; Henry, 1996; Pullaiah and Chennaiah, 1997, 2007 and Suryanarayana and Srinivasa Rao, 2002). The

method of collection of voucher specimens, preservation and mounting of herbarium specimens was done in accordance with the techniques as per Jain and Rao (1977).

Results

The present study revealed unrecorded folk medicinal utilization of 41 plants species belonging to 31 families (Fig. 1-5). These included trees (42%), followed by herbs (26%), climbers (26%) and shrubs (6%). The field observations with folk medicinal claims of the plants species recorded during the study have been presented in table-1.

Important Folk Medicinal Plants of Study Area



Fig 1. *Actinopteris radiatus* (Sw.) Link.



Fig 2. *Holarrhena pubescens* (Buch.-Ham.) Wall. ex G. Don.



Fig 3. *Helicteres isora* Linn.



Fig 4. *Hybanthus enneaspermus* (L.) Muell



Fig 5. *Crinum asiaticum* L.

Table 1: Ethno-pharmacological uses of plants recorded from the tribal groups of Udayagiri forest division.

S. No.	Botanical Name	Habit	Local Name	Part Used	Source of Information	Disease/ Condition	Method of Usage
1.	*Acacia catechu* (L.f.) Willd. (Mimosaceae)	Tree	Kasu chettu	Bark	Chenchus	Scabies	Stem bark paste is applied externally on affected area.
2.	*Acorus calamus* L. (Araceae)	Herb	Vasa	Rhizome	Chenchus	Lack of memory	Root powder, about 500 mg mixed with milk is administered orally once a day for one week to the children as a memory booster.
3.	*Actinopteris radiatus* (Sw.) Link. (Actinopteridaceae)	Herb	Mayurasiki	Whole plant	Chenchus	Rheumatoid arthritis	Paste prepared from whole plant is rubbed on effected joints.
4.	*Adhatoda vasica* Ness Wall. (Acanthaceae)	Shrub	Addasaramu	Root	Yerukulas	Fever	10-15g of root powder is administered orally.
5.	*Alangium salviifolium* (L.f.) Wang. (Alangiaceae)	Tree	Uduga	Root Bark	Chenchus	Snake Bite	Root bark infusion, about 10 ml is taken orally at 2 hours interval entire day as antidote.
6.	*Albizia lebbeck* (L.) Benth. (Mimosaceae)	Tree	Dirisana	Bark	Chenchus	Asthma	Bark powder 10 gm is administered orally for one month.

S. No.	Botanical Name	Habit	Local Name	Part Used	Source of Information	Disease/ Condition	Method of Usage
7.	*Andrographis serpyllifolia* (Rotti ex Vahl.) Wt. (Acanthaceae)	Herb	Jerri mokka	Root	Yanadis	Scolopendra bite.	Root paste, 20 to 30g is administered orally at every 2 hours the entire day and the paste is applied locally on the sight of sting.
8.	*Argemone mexicana* L. (Papaveraceae)	Herb	Balu rakkisa	Latex	Yanadis	Scabies	Latex is applied externally.
9.	*Aristolochia indica* L. (Aristolochiaceae)	Climber	Nalla eswari	Root	Chenchus	Cobra bite	Root paste, about 10 g administered orally for every half an hour up to 12 hours.
10.	*Asparagus racemosus* Willd. (Liliaceae)	Climber	Satavari	Tuberous roots.	Yanadis	Oligogalactea	Raw tuberous roots are consumed as *G*alactagogue.
11.	*Bauhinia racemosa* Lam. (Caesalpiniaceae)	Tree	Arichettu	Bark	Yerukulas	Burning micturation	Bark infusion about 10 ml is taken orally.
12.	*Bombax ceiba* L. (Bombacaceae)	Tree	Mulla buruga	Gum	Yerukulas	Sexual disorders	The gum about 4-7 g is taken daily as aphrodisiac.
13.	*Butea monosperma* (Lam.) Taub. (Fabaceae)	Tree	Moduga	Bark powder	Yerukulas	Piles	Bark powder 5 g is administered orally once a day for one week.

S. No.	Botanical Name	Habit	Local Name	Part Used	Source of Information	Disease/ Condition	Method of Usage
14.	*Calophyllum inophyllum* Linn. (Clusiaceae)	Tree	Ponna chettu	Bark	Chenchus	Pain in testicles	Bark powder is applied externally.
15.	*Ceropegia bulbosa* Roxb. (Asclepiadaceae)	Climber	Batchali dumpa	Tuber	Chenchus	Burning micturation	One tuberous root is taken orally.
16.	*Cissampelos pariera* L. (Menispermaceae)	Climber	Vishaboddi	Root	Chenchus	Snake bite	Oral administration of, about 15 g of root paste and externally keeping the same preparation on the sight of bite works as antidote.
17.	*Corallocarpus epigaeus* Rottl. and Willd. *(Cucurbitaceae*)	Climber	Linga donda	Tuberous root stock	Chenchus	Snake bite	Root powder is administered orally, about 4 g for every 2 hours up to entire day works as antidote for cobra bite.
18.	*Crinum asiaticum* L. (Amaryllidaceae)	Herb	Changlva gadda	Tuberous bulb	Yanadis	Worm infestation	Consuming of bulb, about 5-7 g kills intestinal worms.
19.	*Decalepis hamiltonii* Wight & Arn (Asclepiadaceae)	Climber	Maredu kommulu	Tuberous roots	Chenchus	Burning micturation	Root decoction (20 ml) is administered orally for one week.
20.	*Dioscorea bulbifera* Linn. (Dioscoriaceae)	Climber	Karu.karinda	Bulbils	Yanadis	Skin diseases	External application of paste of bulbils relieves scabies.

S. No.	Botanical Name	Habit	Local Name	Part Used	Source of Information	Disease/ Condition	Method of Usage
21.	*Dioscorea oppositifolia* Var. (Dioscoriaceae)	Climber	Adavidum-pathige	Tuberous roots	Yanadis	Oligospermia	Consuming of Tuberous roots increases sperm count.
22.	*Gmelina asiatica* Linn. (Verbenaceae)	Under shrub	Nela gummadi	Fruit	Yanadis	Dandruff	External application of fruit paste on hair eradicates dandruff.
23.	*Helicteres isora* Linn. (Sterculiaceae)	Tree	Nulidhada	Fruit	Chenchus	Worm infestation	Consuming of fruit powder kills intestinal worms.
24.	*Holarrhena pubescens* (Buch.-Ham.) Wall. ex G. Don. (Apocynaceae)	Tree	Kodisa pala	Bark	Chenchus	Diarrhoea	Consuming of bark powder, about 5 g relieves Diarrhoea.
25.	*Holostemma ada-Kodien* Schultes. (Asclepiadaceae)	Climber	Pala gaddalu	Roots	Chenchus	Sexual disorders	The root is shade dried mixed with bark powder is administered with milk or hot water in dose of 3-5 g a day as aphrodisiac.
26.	*Hybanthus enneaspermus* (L.) Muell. (Violaceae)	Herb	Ratna purusha	Whole plant	Chenchus	Sexual debility	Plant powder is administered orally as aphrodisiac.
27.	*Hygrophila auriculata* (Schum.) Heine. (Acanthaceae)	Herb	Mulla gobbi	Leaves	Chenchus	Jaundice	Consuming of 1 or 2 leaves daily in the morning with cow milk relieves Jaundice.
28.	*Oroxylum indicum* Vent. (Bignoniaceae)	Tree	Dundilamu	Root Bark	Yanadis	Worm infestation	Oral administration of about 10 ml root bark infusion works as Anthelmintic.

S. No.	Botanical Name	Habit	Local Name	Part Used	Source of Information	Disease/ Condition	Method of Usage
29.	*Plumbago zeylanica* Linn. (Plumbaginaceae)	Herb	Tella chitramulamu	Root	Yanadis	Scabies	Root paste is applied externally on affected area.
30.	*Pterocarpus marsupium* Roxb. (Fabaceae)	Tree	Yegisa	Bark and wood	Yanadis	Diabetes	Consuming of infusion prepared from bark and wood controls Diabetes.
31.	*Pueraria tuberosa DC. (Fabaceae)*	Climber	Tella gummadi gadda	Tuberous roots	Yanadis	Impotency	Consuming of root powder daily increases sperm count.
32.	*Saraca asoca* (Roxb.) De Willd. (Caesalpiniaceae)	Tree	Ashoka	Bark	Yerukulas	Bleeding piles	Bark powder (10 g) is given orally once a day for 10 days.
33.	*Semecarpus anacardium* L.f. (Anacardiaceae)	Tree	Nalla jeedi	Gum	Yerukulas	Rheumatic arthritis	Infusion prepared from gum is applied externally on affected areas to get relief from Rheumatic arthritic pain.
34.	*Sida cordifolia* L. (Malvaceae)	Herb	Bala	Root	Yerukulas	Rheumatic arthritis	Root paste mixed with sesame oil heated and applied on affected joints.
35.	*Sphaeranthus indicus Linn. (Asteraceae)*	Herb	Bodasaramu	Inflore-scence	Yerukulas	Cough	Inflorescence powder about 4-5 g is consumed orally which gives immediate relief from cough.

S. No.	Botanical Name	Habit	Local Name	Part Used	Source of Information	Disease/ Condition	Method of Usage
36.	*Spondias pinnata* (L.f.) Kurz. (Anacardiaceae)	Tree	Adivimamidi	Root bark	Yerukulas	Menustrual disorders	Root bark infusion is taken to check red discharge in pregnant ladies.
37.	*Sterculia urens* Roxb. (Sterculiaceae)	Tree	Thapasi	Gum	Chenchus	Diabetes	Oral administration of 4-6 g gum daily controls diabetes.
38.	*Terminalia arjuna* (Roxb.) Wight & Arn. (Combretaceae)	Tree	Tella maddi	Bark	Yanadis	Chest pain, Blood pressure	Bark powder (5-7 g) is given orally daily for one week.
39.	*Tylophora indica* Wt. and Arn. (Asclepiadaceae)	Climber	Meka meyani aaku	Roots	Chenchus	Asthma	One tae spoon full root powder is administered orally 2 times a day.
40.	*Vicoa indica* (Linn.) DC. (Asteraceae)	Herb	Adivi proddutiru-gudu	Root	Chenchus	Antidote for scorpion string.	Oral administration of root powder, about 4g works as antidote for scorpion string.
41.	*Wrightia tinctoria* R.Br. (Apocynaceae)	Tree	Reppala	Bark	Chenchus	Psoriasis	Bark powder mixed with sesame oil is heated and the medicated oil is applied externally to get relief from psoriasis.

Discussion

Medicinal plants play a vital role in the life of human beings by serving good health of mankind. Present study reveals unique utilization of medicinal plants by the tribal groups belonging to Chenchus, Yerukulas and Yanadis or Irulas inhabited in Udayagiri forest division of Nellore district in Andhra Pradesh. In the reported claims of present study, most of the plant parts used for medicinal preparations are leaf, stem bark, root, root bark and gum. All these parts have been reported to be employed in the form of paste, powder, decoction, infusion and also in crude form. Majority of the external applications are for skin diseases, wounds and dandruff. Other external uses reported herein are for arthritis and snake bite. Majority of the internal uses are for conditions like gastro-intestinal disorders, genito- urinary, hepato-biliary, respiratory problems and also as antidotes. Among the reported claims, most of the claims are claimed by Chenchus (20 claims), Yanadis (12 claims) and Yerukulas (9 claims). Many of these claims have been compared with recent literature (Anonymous, 1948-1976, 1992; Hussain *et al.*, 1992; Jain *et al.*, 1991; Rastogi and Mehrotra, 1996-1998; Chetty & Rao, 1989; Hemadari, 1981; Hemadri *et al.,* 1987 & 1988**;** Vijaykumar & Pullaiah, 1998; Nagaraju & Rao, 1990; Balaji Rao *et al*., 1995; Gupta *et al*., 2003, 2005, 2007, 2009; Sudhakar and Rao, 1985; Suryanarayana, 1996; Vedavathy & Mrudula, 1996; Vedavathy and Rao, 1995; Nagaraju, and Rao, 1990 and Nadkarni, 1976) and found that most of the folk medicinal plants are duly reported in the literature, however, their mode of application, ingredients and parts used are different. Therefore, present study represents contemporary uses of medicinal plants for the area investigated.

Conclusion

It can be concluded that information on the majority of these folk preparations in the particular indications may enrich the available ancient traditional as well as contemporary sciences. These species should be studied further to evaluate their efficacy to prove them scientifically for the benefit of common man. This may provide the potential role of ethnobotanical studies in the development of new drugs.

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Pharmacopoeial Standards on *Emblica officinalis* Gaertn. (Aamla) for Harmonization Approach: Review

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Abstract

The dried or fresh mature fruits of *Emblica officinalis* Gaertn. are specified source of drug named 'Aamla'. It is commonly known as Indian gooseberry or Amla. *E. officinalis* is an important medicinal plant in Indian System of Medicines (Ayurveda, Unani and Siddha). It is one of the most important ingredient in Triphala {an Ayurvedic herbal rasayana formula consisting of equal parts of three myrobalans, taken without seed in Amalaki *(Emblica officinalis*), Bibhitaki *(Terminalia bellirica*) and Haritaki *(Terminalia chebula*) }. It is prescribed for improving digestion and assimilation. The drug is also used in famous ayurvedic preparation Chyawanprash which is used for cough, bronchitis, haemoptysis, tuberculosis and as rejuvenating tonic. The drug is highly regarded for varied therapeutic values and enjoys the official status in Unani Pharmacopoeia of India, Ayurvedic Pharmacopoeia of India, Siddha Pharmacopoeia of India, Homoeopathic Pharmacopoeia of India and Indian Pharmacopoeia. Pharmacopoeial monographs on the drug are regulatory standards and mandatory for compliance to ensure the quality, safety and efficacy of the drug. This communication reviews the pharmacopoeial monographs and analytical parameters specified in different pharmacopoeial and non-pharmacopoeial publications with a view on harmonization aspect of quality specifications of *E. officinalis* Gaertn.

Keywords: *Emblica officinalis* Gaertn, Pharmacopoeias, Pharmacopoeial harmonization.

Introduction

The fruits of *Emblica officinalis* Gaertn. (Family Euphorbiaceae) is commonly known as Amla and is widely used as ingredient in the herbal and classical preparation of Unani, Ayurvedic and Siddha system of medicine. The fruit is common ingredient in many Unani and Ayurvedic formulations. *E. officinalis* Gaertn. is a deciduous tree and found natively in India. It is widely cultivated Pakistan, Bangladesh, Ceylon, Malaya, southern China and the Mascarene Islands. Amla literally means 'sour'. The plant species is one of the sacred tree of India. "*Kartik Mahatama* orders the worship of the tree and that a Brahmin couple should a feed under it, whereby all their sins are washed" (Pale, 2006).

Amla is highly nutritious and is an important dietary source of Vitamin C, minerals and amino acids. It raises immunity, it has properties of stimulating the pancreas which secretes insulin for reducing blood sugar. Amla seeds or dried amla is equally invaluable for control of diabetes. It is an excellent antipyretic, antioxidant,

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astringent, laxative, diuretic, aphrodisiac, antacid and haemostatic. It also acts as a booster for physiological systems of the body.

Observations

The review of Pharmacopoeial monographs on herbal drugs published in Unani, Ayurveda, Siddha, Homeopathic and Indian Pharmacopoeias reveal that drug 'Aamla' or 'Emblica' (botanically specified as *Emblica officinalis* Gaertn.) is subjected for regulatory standards in Unani Pharmacopoeia of India, Ayurvedic Pharmacopoeia of India, Siddha Pharmacopoeia of India, Homeopathic Pharmacopoeia of India, Indian Pharmacopoeia, American Herbal Pharmacopoeia and British Pharmacopoeia (Table -1).The non-regulatory quality standards on this drug are also published in Indian Herbal Pharmacopoeia and Quality Standards of Indian Medicinal Plants. (Anonymous, 1940; 1955; 1986; 1998; 2000; 2002; 2007; 2008 a & b; 2011; 2014; 2013 and 2014). The monographs published in these pharmacopoeial and non-pharmacopoeial publications are taken into consideration as per the pharmacopoeial parameters to assess the variability in quality standards of drug. The format of pharmacopoeial monographs in various edition of pharmacopoeias consist varied botanical, physico-chemical and other parameters (Table-2) and pharmacopoeial standards on the drug published in pharmacopoeias are compiled in Table-3 (A & B) and 4. Non-regulatory quality standard published in Indian Herbal Pharmacopoeia and Quality Standards of Indian Medicinal Plants are enumerated in Table-5.

Conclusion

Indian regulatory system on drugs recognizes separate pharmacopoeias for different systems of medicines (Anonymous, 1940). Indian Pharmacopoeia (IP) is the first official pharmacopoeia having its first edition in the year 1955 followed by the publication of other pharmacopoeias viz. Ayurvedic Pharmacopoeia of India (1986), Unani Pharmacopoeia of India (1998), Siddha Pharmacopoeia of India (2008b) and Homoeopathic pharmacopoeia of India (1971). All these Pharmacopoeias provide regulatory standards under Drugs & Cosmetics Act 1940 & Rules thereunder for quality control of drugs of Ayurvedic, Siddha, Unani, Homoeopathic and modern systems of medicine. Pharmacopoeial standards on *E. officinalis* Gaertn. in respect of total ash, acid insoluble ash, alcohol/ethanol soluble extractives and water soluble extractive varies in different pharmacopoeial monographs (Table-6).The description (macroscopic and microscopic powder) to facilitate uniformity in regulatory quality specifications, harmonization of pharmacopoeial standards is very much needed when acceptability of herbal drugs in uniform manner is accelerating (Rai and Sharma, 2014).

Table 1: Pharmacopoeial status of *E. officianalis* Gaertn.

Botanical Name (as specified in Pharmacopoeial Monograph)	Morphological Part specified as drug	Pharmacopoeial/ Monograph Title	Regulatory/Pharmacopoeial References
Emblica officianalis Gaertn.	Fresh and dried mature fruit	Aamla	UPI-I
		Amalaki	API- I
		Aamla	NFUM
		Emblica officinalis	HPI-VIII
		Amalaki	AFI
		Amalaki	IP-2007, IP-2014
		Amla juice powder	IP-2014
		Aamla	UPI-I
		Phyllanthus emblica L. Amla Fruit	American Herbal Pharmacopoeia (AHP) 2011
		Phyllanthus Emblica Pericarp	British Pharmacopoeia (BP) 2013

Abbreviations: API-Ayurvedic Pharmacopoeia of India, UPI-Unani Pharmacopoeia of India, SPI-Siddha Pharmacopoeia of India, HPI-Homeopathic Pharmacopoeia of India, IP-Indian Pharmacopoeia, BP-British Pharmacopoeia, AHP-American Herbal Pharmacopoeia

Table 2: Monograph pattern in various editions of Indian Pharmacopoeias

Sl. No.	Quality Specification	Unani Pharmacopoeia of India, Part-I (UPI) Volume-I	Ayurvedic Pharmacopoeia of India, Part-I (API) Volume-I & VIII	Siddha Pharmacopoeia of India, Part-I (SPI) Volume-I	Homoeopathy Pharmacopoeia of India (HPI) Volume-VIII	Indian Pharmacopeia (IP 2014)
1.	**Pharmacopoeial Title**	√	√	√	√	√
2.	**Definition-Botanical Name (family), Part used as distribution**	√	√	√	Botanical name, Family, Part used, Distribution are under independent headings	“
3.	**Synonyms**	√	√	√	√	√
4.	**Regional Language Name**	√	√	√	Common Names	–
5.	**Description** Macroscopic Microscopic Powder	√	√	√	Description-Macroscopic Microscopic Powder-independent headings	-

Sl. No.	Quality Specification	Unani Pharmacopoeia of India, Part-I (UPI) Volume-I	Ayurvedic Pharmacopoeia of India, Part-I (API) Volume-I & VIII	Siddha Pharmacopoeia of India, Part-I (SPI) Volume-I	Homoeopathy Pharmacopoeia of India (HPI) Volume-VIII	Indian Pharmacopeia (IP 2014)
6.	**Identity, Purity & Strength**	√	√	√	-	-
	Foreign matter	√	√	√	-	√
	Total ash	√	√	√	-	√
	Acid insoluble ash	√	√	√	-	√
	Alcohol/ethanol soluble extractive	√	√	√	-	√
	Water soluble Extractive	√	√	√	-	√
	Loss on drying	-	√	-	-	√
	Heavy metals	-	√	-	-	√
	Microbial contamination	-	√	-	-	√
	Pesticide residues	-	√	-	-	-
	Aflatoxins	-	√	-	-	-
7.	**Assay**	√	√	-	-	√
8.	**Thin Layer Chromatography**	√	√	√	In certain monographs	√

Sl. No.	Quality Specification	Unani Pharmacopoeia of India, Part-I (UPI) Volume-I	Ayurvedic Pharmacopoeia of India, Part-I (API) Volume-I & VIII	Siddha Pharmacopoeia of India, Part-I (SPI) Volume-I	Homoeopathy Pharmacopoeia of India (HPI) Volume-VIII	Indian Pharmacopeia (IP 2014)
9.	**Constituents**	√	√	√	-	
10.	**Properties and Action (as per system of medicine)**	√	√	√	-	Category
11.	**Important Formulations**	√	√	√	-	-
12.	**Therapeutic Uses**	√	√	√	-	-
13.	**Dose**	√	√	√	-	-
14.	**Identification**	-	-	-	√	Macroscopic Microscopic & TLC
15.	**History and authority**	-	-	-	√	-
16.	**Preparation**	-	-	-	√	-
17.	**Heavy metals**	-	-	-	-	√
18.	**Loss on drying**	-	-	-	-	√
19.	**Microbial contamination**	-	-	-	-	√
20.	**Storage**	-	-	-	-	√

Table 3: (A) Comparative account of pharmacopoeial standards on *Emblica officianalis* Gaertn. published in various editions of Indian Pharmacopoeias

Sl. No.	Quality Specification	Unani Pharmacopoeia of India (UPI), Part-I, Volume-I	Ayurvedic Pharmacopoeia of India (API), Part-I, Volume-I	Ayurvedic Pharmacopoeia of India (API), Part-I, Volume-VIII	Ayurvedic Pharmacopoeia of India (API), Part-I, Volume-VIII
1.	**Official Title**	Aamla	Amalaki (Fresh)	Amalaki	Amalaki (Powder)
2.	**Botanical species**	*Emblica officianalis* Gaertn.	*Emblica officianalis* Gaertn.	*Phyllanthus emblica* Linn.	*Phyllanthus emblica* Linn.
3.	**Synonyms**	*Phyllanthus emblica* Linn.	*Phyllanthus emblica* Linn.	*Emblica officianalis* Gaertn.	*Emblica officianalis* Gaertn.
4.	**Morphological part/ Official part**	Fresh mature fruits	Dried mature fruits	Dried pericarp of mature fruits	Powder of dried pericarp of mature fruits
5.	**Description**	I. Macroscopic II. Microscopic III. Powder	I. Macroscopic II. Microscopic III. Powder	I. Macroscopic II. Microscopic III. Powder	I. Macroscopic II. Microscopic
6.	**Identity, Purity & Strength**				
	Foreign matter	2.0 %, Not more than	2.0 %, Not more than	3.0 %, Not more than	3.0 %, Not more than
	Total ash	7.0 %, Not more than	7.0 %, Not more than	7.0 %, Not more than	7.0 %, Not more than
	Acid insoluble ash	2.0%,Not more than	2.0%,Not more than	2.0%,Not more than	2.0%,Not more than

Sl. No.	Quality Specification	Unani Pharmacopoeia of India (UPI), Part-I, Volume-I	Ayurvedic Pharmacopoeia of India (API), Part-I, Volume-I	Ayurvedic Pharmacopoeia of India (API), Part-I, Volume-VIII	Ayurvedic Pharmacopoeia of India (API), Part-I, Volume-VIII
	Alcohol/ethanol soluble extractive	40.0%, Not less than*	40.0%, Not less than*	40.0%, Not less than*	40.0%, Not less than*
	Water soluble extractive	50.0%, Not less than	50.0%,Not less than	50.0%,Not less than	50.0%,Not less than
	Loss on drying	-	-	12.0 %, Not more than	12.0 %, Not more than
	Heavy metals	-	-	Pharmacopoeial limits (Vol. VIII)	Pharmacopoeial limits (Vol. VIII)
	Microbial contamination	-	-	Pharmacopoeial limits (Vol. VIII)	Pharmacopoeial limits (Vol. VIII)
	Pesticide residues	-	-	Pharmacopoeial limits (Vol. VIII)	Pharmacopoeial limits (Vol. VIII)
	Aflatoxins	-	-	Pharmacopoeial limits (Vol. VIII)	Pharmacopoeial limits (Vol. VIII)
7.	**Assay**	-	-	Gallic acid-0.8%, Not less than	Gallic acid-0.8%, Not less than
8.	**Chromatography (TLC/HPTLC/HPLC)**	-	-	TLC/HPTLC profile (Vol. VIII)	TLC/HPTLC profile (Vol. VIII)

*Alcohol (25 percent) soluble extractive

Table 3: (B) Comparative account of pharmacopoeial standards on *Emblica officianalis* Gaertn. published in various editions of Indian Pharmacopoeias

Sl. No.	Quality Specification	Siddha Pharmacopoeia of India (SPI), Part-I, Volume-I	Siddha Pharmacopoeia of India (SPI), Part-I, Volume-I	Homoeopathy Pharmacopoeia of India (HPI) Volume-VIII	Indian Pharmacopeia (IP 2014)
1.	**Official Title**	Nellikkai (Fresh fruit)	Nellivarral (Dried fruit)	Emblica Officianalis (Emb. off.)	Amalaki
2.	**Botanical Species**	*Phyllanthus emblica* Linn.	*Phyllanthus emblica* Linn.	*Emblica officianalis* Gaertn.	*Emblica officianalis* Gaertn.
3.	**Synonyms**	*Emblica officianalis* Gaertn.	*Emblica officianalis* Gaertn.	*Phyllanthus emblica* Linn.	*Phyllanthus emblica* Linn.
4.	**Morphological part/ Official part**	Fresh mature fruits	Dried pericarp of mature fruits	Fruit	Dried pericarp of mature fruits
5.	**Description**	I. Macroscopic II. Microscopic	I. Macroscopic II. Microscopic III. Powder	I. Macroscopic II. Microscopic	I. Macroscopic II. Microscopic
6.	**Identity, Purity & Strength**				
	Foreign matter	2.0 %, Not more than	3.0 %, Not more than	-	3.0 %, Not more than

Sl. No.	Quality Specification	Siddha Pharmacopoeia of India (SPI), Part-I, Volume-I	Siddha Pharmacopoeia of India (SPI), Part-I, Volume-I	Homoeopathy Pharmacopoeia of India (HPI) Volume-VIII	Indian Pharmacopeia (IP 2014)
	Total ash	7.0 %, Not more than	7.0 %, Not more than	-	5.0 %, Not more than
	Acid insoluble ash	2.0%,Not more than	2.0%,Not more than	-	2.0%,Not more than
	Alcohol/ethanol soluble extractive	40.0%, Not less than*	40.0%, Not less than*	-	30.0%, Not less than
	Water soluble extractive	50.0%, Not less than	50.0%, Not less than	-	40.0%,Not less than
	Loss on drying	80.0%, Not less than	-	-	12.0 %,Not more than
	Heavy metals	-	-	-	Pharmacopoeial limits
	Microbial contamination	-	-		Pharmacopoeial limits
	Pesticide residues	-	-	-	-
	Aflatoxins	-	-	-	-
7.	**Assay**	-	-	-	Gallic Acid 1.0%, Not less than
8.	**Chromatography (TLC/HPTLC/HPLC)**	TLC profile	TLC profile	-	TLC profile

Table 4: Comparative account of pharmacopoeial standards on *E. officianalis* Gaertn. published in other Pharmacopoeias

Sl. No.	Quality Specification	American Herbal Pharmacopoeia (AHP) 2011	British Pharmacopoeia (BP) 2013
1.	**Official title**	Phyllanthus emblica L. Amla Fruit	Phyllanthus Emblica Pericarp
2.	**Botanical species**	*Phyllanthus emblica* L.	*Phyllanthus emblica* L.
3.	**Morphologicalpart/ Official part**	Fruit and seed	Dried pericarp of mature fruits
4.	**Description**	I. Macroscopic II. Microscopic III. Powder	I. Macroscopic II. Microscopic III. Powder
5.	**Identity, Purity & Strength**		
	Foreign matter	-	5.0%, Not more than
	Total ash	-	7.0%, Not more than
	Acid insoluble ash	-	-
	Alcohol/ethanol soluble extractive	-	15.0%, Not more than
	Water soluble extractive	-	50.0%, Not more than
	Loss on drying	-	10.0%, Not more than
	Heavy metals	-	-
	Microbial contamination	-	-
	Pesticide residues	-	-
	Aflatoxins	-	-
6.	**Assay**	-	Tannins (expressed as pyrogallol) - 6.0%, Not less than
7.	**Chromatography (TLC/HPTLC/HPLC)**	-	HPLC profile

Table 5: Comparative account of pharmacopoeial standards on *E. officianalis* Gaertn. published on non-regulatory quality standards

Sl. No.	Quality Specification	Indian Herbal Pharmacopoeia (IHP) 2002	Quality Standards of Indian Medicinal Plants (QCIMP) 2010
1.	**Official title**	Emblica Officinalis	*Emblica officianalis* Gaertn. (Amalaki)
2.	**Botanical species**	*Emblica officianalis* Gaertn.	*Emblica officianalis* Gaertn.
3.	**Synonyms**	*Phyllanthus emblica* Linn	*Phyllanthus emblica* Linn
4.	**Morphological part/ Official part**	Dried and fresh fruit	Dried and fresh fruit
5.	**Description**	I. Macroscopic II. Microscopic	I. Macroscopic II. Microscopic III. Powder
6.	**Identity, Purity & Strength**		
	Foreign matter	3.0 %, Not more than	Nil
	Total ash	5.0 %, Not more than	1.0 %, Not more than (Fresh fruit) 12.0 %, Not more than (Dried fruit)
	Acid insoluble ash	2.0%,Not more than	0.05%, Not more than (Fresh fruit) 2.0%, Not more than (Dried fruit)
	Alcohol/ethanol soluble extractive	40.0%, Not less than*	10.0%, Not less than (Fresh fruit) 31.0%, Not less than (Dried fruit)
	Water soluble extractive	40.0%, Not less than	11.0%, Not less than (Fresh fruit) 46.0%, Not less than (Dried fruit)
7.	**Assay**	Quantification not given	Gallic Acid ranges - 0.015 to 0.022
8.	**Chromatography (TLC/HPTLC/HPLC)**	-	TLC/HPTLC profile

*Alcohol (25 percent) soluble matter.

Table 6: Variation in standards in respect of pharmacopoeial standards

Sl. No.	Quality Specification	Unani Pharmacopoeia of India (UPI), Part-I, Volume-I	Ayurvedic Pharmacopoeia of India (API), Part-I, Volume-I & VIII	Siddha Pharmacopoeia of India (SPI), Part-I, Volume-I	Homoeopathy Pharmacopoeia of India (HPI) Volume- VIII	Indian Pharmacopeia (IP 2014)
1.	Foreign matter	2.0 %, Not more than	2.0 %, Not more than	2.0 %, Not more than	-	3.0 %, Not more than
2.	Total ash	7.0 %, Not more than	7.0 %, Not more than	7.0 %, Not more than	-	5.0 %, Not more than
3.	Acid insoluble ash	2.0%, Not more than	2.0%, Not more than	1.0%, Not more than	-	2.0%, Not more than
4.	Alcohol/ethanol soluble extractive	40.0%, Not less than	40.0%, Not less than (Vol. VIII)	40.0%, Not less than	-	30.0%, Not less than
5.	Water soluble extractive	50.0%, Not less than	50.0%, Not less than	50.0%, Not less than	-	40.0%, Not less than
6.	Microbial contamination	-	Pharmacopoeial limits (Vol. VIII)	-	-	Pharmacopoeial limits
7.	Pesticide residues	-	Pharmacopoeial limits (Vol. VIII)	-	-	-
8.	Aflatoxins	-	Pharmacopoeial limits (Vol. VIII)	-	-	-

Pharmacopoeial standards on *Emblica officinalis* Gaertn. incorporated in various pharmacopoeias are required to be harmonized with the monographs appeared in other contentmpoarory pharmacopoeias. The quality of herbal drugs is always prime issue and dealt with regulatory provisions of pharmacopoeial monographs. The harmonized pharmacopoeial monographs will be yardstick to ensure the quality, safety and efficacy of herbal drugs without any ambiguity.

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Ethnomedicinal Uses of Some Pteridophytic Species From North India

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Abstract

During ethnobotanical explorations of different forest divisions from 1998 to 2005 in Uttar Pradesh and Uttarakhand states of northern India, the authors have recorded a large number of information regarding the uses of higher plants for medicine. This field study has also yielded information on some 11 pteridophytic species that are widely used by the tribal and other rural communities of the area as folk medicines for treatment of various disorders. A comparison with relevant literature revealed that many uses are hitherto new or imperfectly known. Hence, an attempt has been made to highlight ethnomedicinal value of such species in the present report. It lists eleven species of medicinal ferns. Each entry has been provided with correct botanical and prevalent local names, habit and habitat, claimed medicinal use (s) and other observations.

Keywords: Folk medicine, Medicinal ferns, Pteridophytes, Uttar Pradesh, Uttarakhand.

Introduction

Pteridophytes, which include the ferns and fern allies, are a group of ancient vascular plants with worldwide distribution. They are represented by about 305 genera, comprising more than 10,000 species all over the world (Singh and Viswanathan, 1996; Parihar and Parihar, 2006). Pteridophytic vegetation greatly contributes to the earth's plant diversity. These primitive vascular plants form an important and dominant component of many plant communities. The India has a rich and varied pteridophytic flora due to its diversified topography, variable climatic conditions and soil types. About 191 genera and more than 1000 species of pteridophytes have been reported from different regions of India (Dixit, 1984; Dixit and Vohra, 1984; Bir, 1977, 1992; Chandra, 2000). Majority of the members prefers shady and hygroscopic conditions for prolific growth (Bir, 1993), but also occur throughout in an extra ordinary range of habitats (Shankar and Khare, 1994; Vasudeva, 1999; Srivastava, 2007; Kumar and Kaushik, 1999). Like the other group of plants, pteridophytes are also show medicinal value. People throughout the world have been utilizing many ferns as medicine for the treatment of different diseases and conditions since ancient times. Caius (1935) has described the medicinal and poisonous ferns of India. Besides, Das (2003), Kaushik and Dhiman (1995), Manickam (1999), Nayar (1959), Singh (1999), Watt (1959) have also described medicinal uses of some pteridophytes of India. Furthermore, Dhiman (1998), Dixit and Singh (2004), Kumari *et al.,* (2011), Padala (1998), Puri (1970), Srivastava, (2007a), Trivedi (2002), Vyas and Sharma (1998), have much

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contributed to the knowledge of ethnobotany and medicinal uses of pteridophytes. A number of authors have also reported many medicinal ferns used by the rural and tribal communities in different parts of northern India (Gaur and Bhatt, 1994; Joshi, 1997; Kholia and Punetha, 2005; Sharma, 1985; Siddiqui and Husain, 1991; Singh *et al.*, 1989; Singh and Roy, 1986; Srivastava, 2007b; Srivastava et al., 2013; Upreti *et al.*, 2009; etc.). In the present paper we have reviewed and analysed some useful information on 11 species of pteridophytes that are used in folk medicine and very well known to indigenous communities of various forests of this region of the country. This contribution is an addition to the above reports.

Material and Methods

Plant materials for the present study were obtained from the data recorded during ethnobotanical explorations of various forest divisions like Kashi, Moradabad, Pilibhit, Shivalik of Uttar Pradesh and Haldwani, Kalsi, Nainital, Pithoragarh, Ramagar of Uttarakhand. Fieldwork was carried out in different seasons from 1998 to 2005. The data were collected according to the methodology of Singh and Ali (1992). The identification of some specimens was finally confirmed by matching with the specimens at the herbarium of Forest Research Institute, Dehradun (DD). For compilation of data, all medicinally important pteridophytic species are arranged in alphabetic order by their botanical names with respective family in parenthesis, local name, locality and voucher specimen number, habit and habitat, ethnomedicinal use (s). This is followed by a remark on presence of major chemical constituents. All the specimens were preserved in the herbarium of Survey of Medicinal Plants Unit, Regional Research Institute of Unani Medicine, Aligarh (U.P.), India.

Observations

1. *Adiantum capillus-veneris L.* (Adiantaceae)

Local name:	*Hansraj*
Locality and F. B. No.:	Shivalik (9171), Moradabad (5225)
Habit and habitat:	A small fern with short-creeping, dark brown, densely scaly rhizome. Plants grow in crevices of shady rocks and forms dense clusters.
Ethnomedicinal use (s):	About 20g of dried fronds are boiled in 100ml of water. This decoction with powder of few black peppers and little honey is given two times a day for 30-45 days in asthma.

Chemical constituents:	Tannin, kaempferol, quercetol, astragalin, lutelol, rutin, triterpinoid, isoquercitrin, nicotiflorin, querciturone and flavonoids naringenin, hesperidin sulphuretin and genistein.

2. *Adiantum incisum* Forsk. (Adiantaceae)

Local name:	*Hansraj, Hansavati*
Locality and F. B. No.:	Pilibhit (5942)
Habit and habitat:	A small terrestrial herb. Found in shaded localities in crevices of rocks in the forest.
Ethnomedicinal use (s):	Frequent chewing of fresh fronds is recommended in stomatitis.
Chemical constituents:	Adiantone, isoadiantone, triterpenes-fernene, hentriacontane, hentriacontanone, â-sitosterol.

3. *Adiantum philippense* L. (Adiantaceae)

Local name:	*Chandni Buti*
Locality and F. B. No.:	Haldwani (6336)
Habit and habitat:	A tufted fern, 8-22mm in size, densely covered with fibrous root, scales and leaf bases. Commonly grows along the roadsides in the forest under moist situation in low mountainous region.
Ethnomedicinal use (s):	Fresh fronds with leaves of 'kasni' *(Cichorium intybus* L.) in equal quantities are boiled in water and liquid strained. One cup of the resulting decoction is taken twice daily for irregular menses.
Chemical constituents:	Flavanoids, hesperidin and sulfuretin.

4. *Ampelopteres prolifera* Copeland (Thelypteridaceae)

Local name:	*Lukra*
Locality and F. B. No.:	Pilibhit (5939)
Habit and habitat:	A terrestrial fern. Fronds erect and tufted. Commonly found in moist and shady places.
Ethnomedicinal use (s):	Frond paste is used as antipyretic.
Chemical constituents:	Protein, steroids, triterpinoids, flavones, flavonoides, sugars.

5. *Cheilanthes farinosa* (Forsk.) Kaulf. (Cheilanthaceae)

Local name:	*Chhapa Ghans*
Locality and F. B. No.:	Kalsi (9648)
Habit and habitat:	Erect herb, about 8cm high. Fronds white beneath. Abundantly grows in dry situations and exposed places.
Ethnomedicinal use (s):	Brown stipe is used by females as nose and ear studs. In cases of cows, dried powder of the frond is sprinkled externally on prolapsed uterus and then the uterus is pushed inside.
Chemical constituents:	Cheilanthatriol, cheilarinosin, kaempferol, quercetin, flavonoides, naringenin and dihydroquercetin and phenols.

6. *Diplazium esculentum* (Retz.) Sw. (Athyriaceae)

Local name:	*Leungra*
Locality and F. B. No.:	Nainital (7197)
Habit and habitat:	A large fern with an erect stout caudexes. Commonly found on damp and marshy places.
Ethnomedicinal use (s):	Young shoots are cooked and taken for indigestion.
Chemical constituents:	Protein, vitamin B, iron, calcium phosphorus, steroids, triterpenoids, flavonoides, flavones, sugar.

7. *Equisetum arvense* L. (Equisetaceae)

Local name:	*Hadjoran*
Locality and F. B. No.:	Pithoragarh (8097)
Habit and habitat:	Herb with jointed and hollow stems. Found along ditches.
Ethnomedicinal use (s):	Stem paste mixed with powdered alum is plastered around the fractured limb. Splints and bandages are used to hold the bones and plaster in position.
Chemical constituents:	3-methoxy pyridine, nicotine, palustine, thymine, dimethyl sulphone iso-quercitrin, galiteolin, equisetrin, equisetonin, aconitic acid, kaempferol, quercetin, epigenin, vitamin c, lipids, sterols.

8. *Equisetum ramosissimum* Desf. (Equisetaceae)

Local name: *Joratora, Hadjor, Sarset*

Locality and F. B. No.: Moradabad (5246), Pilibhit (5933), Shivalik (9161); Nainital (7150), Haldwani (6150).

Habit and habitat: A plant with creeping rhizome and ribbed as well as jointed stems. Found in shady or gravely soil along stream and riverbanks in moist or exposed situations.

Ethnomedicinal use (s): A paste of the plant is mixed with little slaked lime and plastered around the fractured limb after setting the bones right. The paste of the aerial parts is given orally to expel small kidney stones. Fresh juice of aerial parts is applied on burns for healing and to prevent scar. Plant juice is used as ear drops for earache.

Chemical constituents: Phenols.

9. *Helminthostachys zeylanica* (L.) Hook. f. (Helminthostachyaceae)

Local name: *Kamraj, Kamrajvati*

Locality and F. B. No.: Pilibhit (5939), Haldwani (6431), Ramnagar (6602)

Habit and habitat: A terrestrial and perennial fern with creeping rhizomes. It flourishes well as undergrowth, chiefly in the forest of Sal. It is restricted to Tarai region of Uttar Pradesh and Uttarakhand. This is an endangered species.

Ethnomedicinal use (s): Rhizomes with few grains of black peppers are ground to make a fine paste. About 10g of this paste are given with milk two times a day, for 15 days in leucorrhoea. It is also used as nutritive tonic for impotency.

Chemical constituents: Ugonins, Stigmasterol, fucosterol and dulcitol.

10. *Lygodium flexuosum* (L.) Sw. (Lygodiaceae)

Local name: *Kalijar*

Locality and F. B. No.: Pilibhit (5979)

Habit and habitat: A terrestrial climbing fern. Commonly grows on bushes and trees or trailing on the ground along the edges of forest in gravelly and sandy soil.

Ethnomedicinal use (s):	Fresh root is rubbed on a stone and the paste thus obtained is given with mother's milk to the infants as an anti diarrhoeal agent. Root paste is mixed with powder of black peppers and given for cough.
Chemical constituents:	Tryptophan, tryptamine, indole-3-acetic acid, -3-propionic acid, -3-butyric acid and 3-acetonitrile.

11. *Selaginella bryopteris* (L.) Bak. (Selaginellaceae)

Local name:	*Pattharchati*
Locality and F. B. No.:	Kashi (7809)
Habit and habitat:	Lithophytic herb up to 15cm high that occurs on rock boulders and forming thick, green carpet during rainy season. Leaves curled up in dry weather but retain original colour and shape if dipped upside down in water for some time.
Ethnomedicinal use (s):	About 12-15g of the leaf paste are given orally twice daily for one month to treat spermatorrhoea.
Chemical constituents:	Hexoses and proteins.



Fig.1 *Helminthostachys zeylanica* (L.) Hook. f.



Fig.2 *Cheilanthes farinosa* (Forsk.) Kaulf.

Discussion

This communication provides a report on ethnomedicinal uses of some important species of pteridophytes revealed by the indigenous people belonging to different forest divisions of Uttar Pradesh and Uttarakhand in northern India. The uses were mostly related to the disorders of digestive, integumentary, musculoskeletal, respiratory and urino-genital systems. The data are authentic and obtained from the knowledgeable village elders and local healers. Leaves are the most commonly utilized plant part in herbal preparations which are used internally in the form of decoction and paste. Data on medicinal uses were analyzed and compared with relevant literature (Anonymous, 1948-1976; Benjamin and Manickam, 2007; Benniamin, 2011; Bharti, 2011; Chopra *et al.*, 1956; Jain, 1991; Kirtikar and Basu, 1935; Kumari *et al.*, 2011; Nadkarni, 1954; Sharma, 2002; Parihar, and Parihar, 2006; Rout, et al., 2009; Singh and Singh, 2012; Upreti *et al.*, 2009; Watt, 1889-1892) and it was found that uses of some species were similar to information already published in the literature. However, majority of these claims are new and imperfectly known and enrich our existing traditional knowledge on medicinal ferns.

Reported species deserve accurate scientific screening and evaluation to demonstrate the effectiveness, safety and establishment of proper dosages. As, these claims are based on ancestral knowledge and empiric experience. Moreover, many pteridophytes contain poisonous substances and carcinogenic factors. A well-known true Maidenhair fern *(Adiantum capillus-veneris*) is not suitable for pregnant women as it is known to be a contraceptive. An excessive dose of the fern can lead to muscular weakness, coma and even injury to the eyes. Such studies on toxicity, pharmacological actions and chemical constituents are very essential to analyse the chemicals of these plants and the implication on health.

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Standardization of Unani Drug – Jawarish Usquf

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Abstract

The drug Jawarish Usquf is therapeutically used in the ailments of Munaqqi-e-Asab, Laqwa and Qulanj. Jawarish Usquf was prepared in three different batches as per the guidelines of NFUM (Part-IV). Present study was aimed to evaluate the powder microscopical studies to identify the raw drugs present in the formulation, physico-chemical data to lay down pharmacopoeial standards, TLC to develop the fingerprints and WHO parameters to ascertain quality of the drug. Powder microscopical studies showed the presence of numerous starch grains, non-lignified septate fibres, reticulate vessels (Zanjabeel); fibres lignified not over 30µ breadth, stone cells horse shoe shaped (Darchini); large mesocarpic parenchyma cells with corner thickening (Aamla); pollen grains tetrahedral upto 20ì, fragments of anther wall (Qaranfal); tracheidal cells with scalariform thickening upto 50µ (Bisfayej); endosperm cells filled with starch grains and crystalloid proteins (Jauzbuwa); perisperm cells with angular walls filled with starch grains (Filfil Siyah); group of bulbous perisperm cells packed with starch grains (Heel Kalan); vessels with pitted thickening; rosette of calcium oxalate crystals (Turbud). The physico-chemical data showed that the drug contains moisture (18.51%), total ash (0.69%), acid in-soluble ash (0.022%) solubility in alcohol (25.46%) and water (64.55%). TLC study showed various spots at 254nm, 366nm and visible light (V-S reagent). The quality control study revealed the absence of microbial load, aflatoxins, heavy metal and pesticide residues. The evaluated standards will be much useful for laying down the pharmacopoeial standards of Jawarish Usquf and also in providing the quality medicine to the needy mass.

Keywords: Jawarish Usquf, Physico-chemical, TLC, and WHO parameters

Introduction

India having a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. Medicinal plants are the major part of these traditional medicines. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of traditional medicines in the healthcare. The plant species mentioned in the ancient texts and other Indian systems of medicine may be explored with the modern scientific approaches for better leads in the healthcare. Standardization of herbal formulations is essential in order to assess the quality of drugs (Yadav & Dixit, 2008; Bandaranayake, 2006; Myers and Cheras, 2004).

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The microscopic characters of each ingredient used in the formulations are very difficult to identify and also some time there are overlapping with the character of other ingredient. Pharmacognostical study of a drug is an essential requirement to establish the presence of each ingredient in the formulations. Quality assurance of herbal medicine is an important factor and basic requirement for herbal drug industry and for other drug development organizations. Due to lack of standard operating procedure and quality control methods, there are batch to batch variations in the same formulation as well as variation among the same formulation procured from different sources.

The drug Jawarish Usquf (Anonymous, 2006) is one of the classical herbal Unani compound formulations. It is therapeutically useful in the ailments of Munaqqi-e-Asab (Nervine tonic), Laqwa (Facial paralysis) and Qulanj (Colic). The preparation of the drug Jawarish Usquf is based on traditional methods in accordance with the procedure given in NFUM, Part-IV (Anonymous, 2006) and it was prepared in three different batches.

The present study is an attempt to prepare and standardized the herbal formulation using pharmacopoeial studies such as organoleptic characters, microscopical, physico-chemical, TLC and quality control parameters.

Material and Methods

Ingredients Authentications

The raw drugs namely Zanjabeel, Darchini, Aamla Munaqqa, Qaranful, Bisfayej, Jauzbuwa, Filfil Siyah, Heel Kalan, Saqmonia, Turbud and Qand Safaid of the formulation were procured from raw drugs dealers of Chennai. These raw drugs were authenticated as per pharmacopoeial and other official standards (Anonymous, 2007 & 2008).

Drug Preparation

The drug was prepared in different batches at Laboratory scale as per the ingredients composition and guidelines of NFUM, Part – IV (Anonymous, 2006) (Table 1). Take the required quantity of all the ingredients of pharmacopoeial quality. The raw drugs (1 to 2 and 4 to 10) were cleaned, dried, powdered and sieved through 80 mesh and kept separately. Aamla Munaqqa was grinded and made into paste and kept separately. Dissolve the specified quantity of sugar (11) in 375ml of water on slow heat. Add 0.1% citric acid and made the quiwam of 74% consistency then add 0.1% sodium benzoate, add the paste of aamla and mixed thoroughly. The quiwam was re-corrected to 77% consistency. The container was removed from fire while the quiwam is in hot add the mixed powders of ingredient number 1 to 2 and 4 to 10 and mixed thoroughly to prepare the

homogenous product. Allowed to cool to room temperature and packed it in tightly closed containers to protect from light and moisture.

Pharmacopoeial Standards

The pharmacopoeial studies such as organoleptic characters, microscopical, physic-chemical, TLC and quality control parameters were carried out:

i. Organoleptic Evaluation: Organoleptic evaluation refers to evaluation of formulation by colour, odour, taste, texture etc. The organoleptic characters of the samples were carried out based on the method described by Siddique *et al.* (1995).

ii. Powder microscopical studies: The drug sample (5g) was weighed and mixed with 50ml of water in a beaker with gentle warming, till the sample completely dispersed in water. The mixture was centrifuged and decanted the supernatant. The sediment was washed several times with distilled water, centrifuged again and decanted the supernatant. A few mg of the sediment was taken and mounted in glycerine. A few mg was taken in watch glass and added few drops of phloroglucinol and concentrated hydrochloric acid, mounted in glycerine. The salient features of the drug were observed in different mounts (Wallis, 1997 and Johansen, 1940).

iii. Physico-chemical analysis: The moisture content at 105°C, ash values, solubility in water and alcohol, pH values, bulk density and sugar content etc., are the useful tools in standardisation of a herbal products as per standards method (Anonymous, 1987 and 1998).

iv. Thin layer chromatography: The drug samples (2g) were soaked in chloroform and alcohol separately for 18 hours, refluxed for ten minutes on water bath and filtered. The filtrates were concentrated on water bath and made up to 5ml in a standard flask separately and carried out the TLC studies (Wagner *et al*., 1984).

v. Other quality control parameter: The usage of herbal products along with higher safety margins, WHO has taken necessary step to ensure quality control parameters with the modern techniques and application of suitable standards. The microbial load and heavy metal parameters were carried out as per the WHO guidelines (Anonymous, 1998). Aflatoxins and pesticide residues were carried out by standard methods (Anonymous, 1997 and 2005).

Results and Discussion

Organoleptic characters: Jawarish Usquf is a brown semi-solid product with sweetish bitter in taste.

Microscopical observations: Isolated starch grains, simple oval to round shaped measuring upto 70ì, hilum eccentric, lamellae distinct; non-lignified septate fibres upto 50ì, reticulate vessels and fragments of reticulate vessels upto 70ì (Zanjabeel); fibres thick walled lignified with striated walls and narrow lumen of length upto 1000µ and breadth not over 30µ, stone cells with horse shoe shaped thickenings upto 100µ (Darchini); epidermal cells in surface view with uniformly thick walled cells containing silica crystals and with occasional paracytic stomata, mesocarpic parenchyma cells with large irregular thick walled cells showing corner thickening (Aamla); pollen grains tetrahedral spherical biconvex measuring upto 20ì in diameter, parenchyma cells with schizolysigenous oil glands, fragments of anther wall in surface view (Qaranfal); tracheidal cells with scalariform thickening upto 50µ (Bisfayej); endosperm cells in surface view with numerous starch grains and crystalloid proteins, each crystalloid proteins upto 40µ, perisperm cells in surface view filled with reddish brown contents (Jauzbuwa); perisperm cells isolated or in groups with angular walls filled with simple and compound starch grains and minute calcium oxalate crystals, stone cells polygonal upto 60µ interspersed among parenchyma cells with circular lumen (Filfil Siyah); group of bulbous perisperm cells packed with starch grains and tiny prismatic crystal of calcium oxalate, elongated thin walled parenchyma cells from aril tissue, orange coloured thick walled sclerenchyma cells in surface view (Heel Kalan); vessels with pitted thickening (simple pits) of length upto 400µ, breadth upto 200µ; rosette of calcium oxalate crystals upto 60µ, starch grains simple and compound; simple starch grains elliptical to spherical with central cleft hilum upto 25µ, compound starch grains 2 to 4 grains unite; medullary ray parenchyma cell filled with starch grains (Turbud) (Fig.1).

Physico-chemical: The physico-chemical analysis such as moisture content obtained in the drug was 18.51%. The alcohol soluble extractive (25.46%) might be due to the extraction of polar chemicals constituents and the water soluble extractives (64.55%) indicate the presence of inorganic constituents. The obtained data are shown in Table - 2.

Thin Layer Chromatography analysis: The chloroform and alcohol extract of all the three batch samples showed identical spots in UV – 254nm and 366nm ranges and the R_f values of both the extracts are shown in Table-3 and 4. The plates were dipped in vanillin-sulphuric acid and heated at 105ºC till appeared coloured spots.

Quality control parameters: The evaluated quality control parameters such as microbial load and heavy metals were found within the permissible limit in the drug shown in Table - 5 and 6. The other parameters like aflatoxins B_1, B_2, G_1 and G_2 and pesticide residues were not detected from the drug samples shown in Table - 7 and 8.

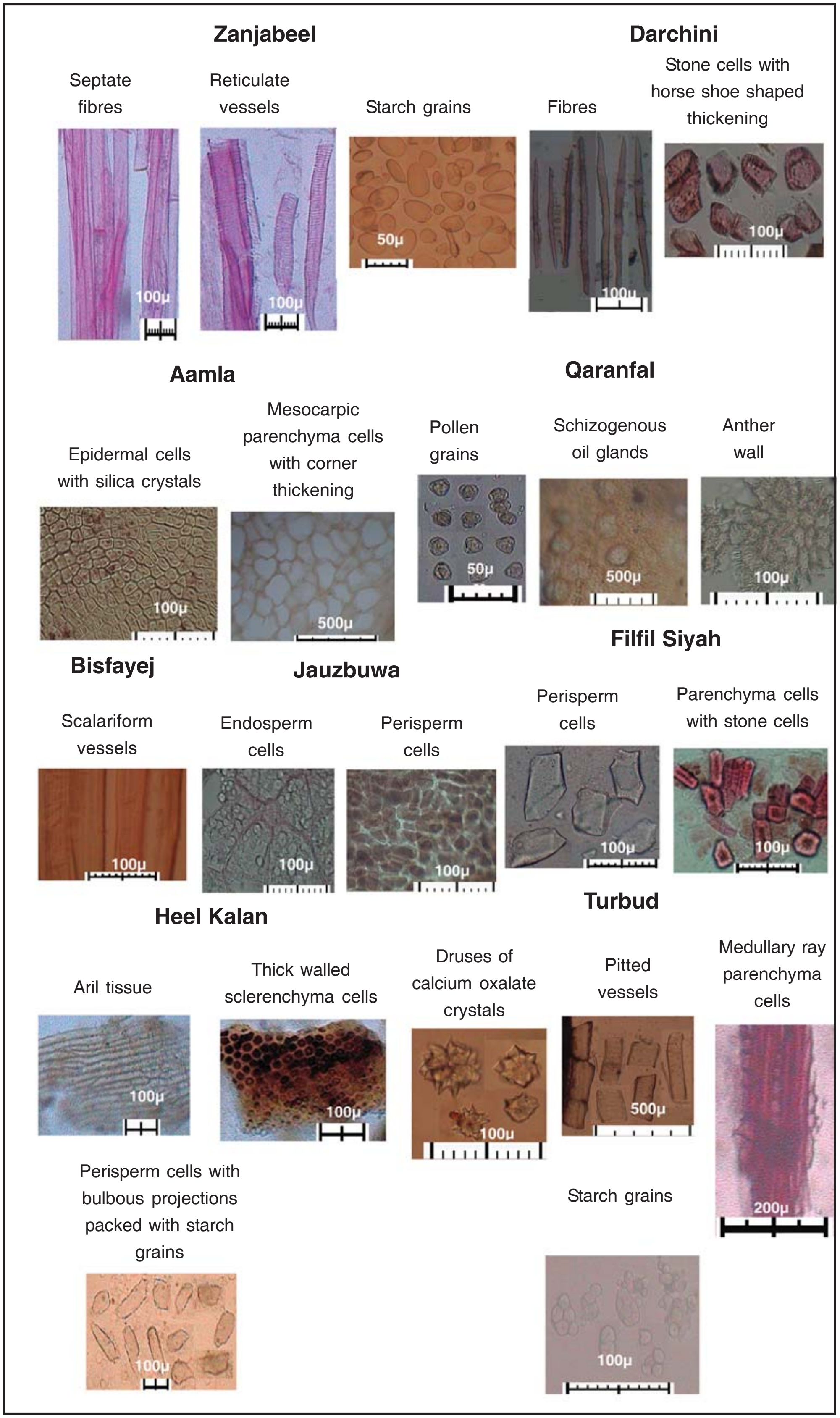


Fig. 1: Powder microscopy of Jawarish Usquf

Table 1: List of Ingredients in Jawarish Usquf

S.No.	Unani name	Unani Name	Part used	Quantity
1.	Zanjabeel Khushk UPI-I	*Zingiber officinale* Rosc	Rhizome	10 g.
2.	Darchini UPI-I	*Cinnamomum zeylanicum* Blume.	Inner stem bark	10 g.
3.	Aamla Munaqqa UPI-I	*Emblica officinalis* Gaertn.	Fruit	10 g.
4.	Qaranful UPI-I	*Syzygium aromaticum* (L.) Merr. L M Perry	Flower bud	10 g.
5.	Bisfayaj UPI-II	*Polypodium vulgare* Linn	Rhizome	10 g.
6.	Jauzbuwa UPI-I	*Myristica fragrans* Houtt.	Endosperm	10 g.
7.	Filfil Siyah UPI-IV	*Piper nigrum* Linn.	Fruit	15 g.
8.	Heel Kalan UPI-IV	*Ammomum subulatum* Roxb.	Fruit	15 g.
9.	Saqmonia	*Convolvulus scammonia* L.	Resin	15 g.
10.	Turbud UPI-V	*Operculina turpethum* Linn.	Root	15 g.
11.	Qand Safaid	Sugar	—	305 g.

Table 2: Physico-chemical parameters

Parameters	Batch Number		
	I	II	III
Extractives			
Alcohol soluble matter	25.21%; 25.40%; 25.52%	25.64%; 25.72%; 25.84%	25.18%; 25.24%; 25.36%
Water soluble matter	64.14%; 64.20%; 64.32%	64.44%; 64.56%; 64.76%	64.72%; 64.88%; 64.96%
Ash			
Total ash	0.71%; 0.74%; 0.81%	0.58%; 0.64%; 0.86%	0.54%; 0.59%; 0.77%
Acid insoluble ash	0.018%; 0.022%; 0.027%	0.013%; 0.017%; 0.025%	0.017%; 0.026%; 0.031%
pH values			
1% Aqueous solution	5.14; 5.23; 5.44	5.17; 5.31; 5.39	5.09; 5.24; 5.49
10% Aqueous solution	4.34; 4.39; 4.60	4.28; 4.31; 4.53	4.37; 4.42; 4.53
Sugar estimation			
Reducing sugar	24.22%; 22.32%; 22.39%	24.12%; 24.25%; 24.37%	24.17%; 24.27%; 24.33%
Non-reducing sugar	21.38%; 21.53%; 21.60%	21.57%; 21.70%; 21.81%	21.49%; 21.62%; 21.79%
Moisture	18.17%; 18.21%; 18.38%	18.35%; 18.44%; 18.58%	18.68%; 18.83%; 18.96%
Bulk Density	1.2805; 1.2875; 1.907	1.2799; 1.2898; 1.2954	1.2785; 1.2856; 1.2897

Table 3: *Rf* Values of chloroform extract

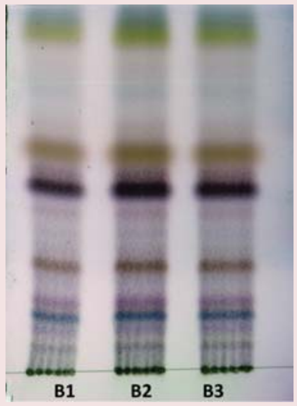
Solvent system Toluene: Ethyl acetate (9: 1)	Rf Values		
	UV 254nm	**UV 366nm**	**V. S. Reagent**
 **Fig. 2 V.S. Reagent**	0.89 Brown	0.93 Brown	0.92 Yellowish green
	0.73 Pink	0.74 Fluorescent blue	0.82 Grey
	0.61 Pink	0.66 Fluorescent blue	0.73 Grey
	0.52 Pink	0.51 Light blue	0.58 Brown
	0.46 Pink	0.45 Blue	0.48 Violet
	0.41 Light pink	0.33 Blue	0.41 Grey
	0.28 Pink	0.20 Blue	0.26 Brown
	0.25 Light pink		0.18 Violet
	0.13 Pink		0.13 Pink

Table 4: *Rf* Values of alcohol extract

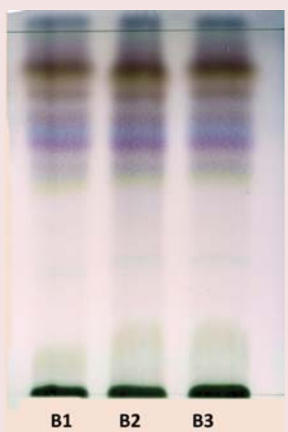
Solvent system Toluene: Ethyl acetate (6: 4)	Rf Values		
	UV 254nm	**UV 366nm**	**V. S. Reagent**
 **Fig.- 3 V. S. Reagent**	0.93 Grey	0.92 Yellow	0.87 Violet
	0.83 Pink	0.85 Fluorescent blue	0.85 Brown
	0.66 Light pink	0.79 Blue	0.81 Violet
	0.59 Light pink	0.72 Blue	0.71 Grey
	0.55 Pink	0.67 Blue	0.64 Violet
	0.49 Light pink	0.56 Light blue	0.56 Yellowish green
	0.29 Light pink	0.49 Blue	0.32 Grey
	0.18 Light pink	0.19 Light blue	0.31 Light grey

Table 5: Analysis of Microbial load

S.No.	Parameter Analyzed	Results	WHO Limits
1	Total Bacterial Count	200 CFU / gm	10^5 CFU / gm
2	Total Fungal Count	200 CFU / gm	10^3 CFU / gm
3	Enterobacteriaceae	Absent	10^3 CFU / gm
4	*Salmonella* spp.	Absent	Absent
5	*Staphylococcus aureus*	Absent	Absent

Table 6: Estimation of Heavy Metals

S.No.	Parameter Analyzed	Results	WHO & FDA Limits
1	Arsenic	Not detected	10 ppm
2	Cadmium	Not detected	0.3 ppm
3	Lead	Not detected	10 ppm
4	Mercury	Not detected	1.0 ppm

Table 7: Estimation of Aflatoxins

S.No.	Aflatoxins	Results
1	B1	Not Detected
2	B2	Not Detected
3	G1	Not Detected
4	G2	Not Detected

Table 8: Analysis of Pesticide Residues

S.No.	Pesticide Residues	Results
1	**Organo Chlorine group**	Not Detected
2	**Organo Phosphorus group**	Not Detected
3	Acephate	Not Detected
4	Chlordane	Not Detected
5	Dimethoate	Not Detected
6	Endosulphan	Not Detected
7	Endosulfan	Not Detected
8	Endosulfon	Not Detected
9	Ethion	Not Detected
10	Endosufon sulphate	Not Detected
11	Fenthion	Not Detected
12	Heptachlor	Not Detected
13	Lindane	Not Detected
14	Methoxychlor	Not Detected
15	Phorate sulfoxide	Not Detected
16	Phorate sulfone	Not Detected

Conclusion

In the present study the Unani formulation Jawarish Usquf has been standardized by modern scientific quality control measures. The results obtained from these pharmacopoeial parameters could be used to analyse the formulation and to check the quality and batch-to-batch variations.

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Physicochemical Standardization of *Habbe Mubarak:* A Unani Compound Formulation

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Abstract

Unani System of Medicine (USM) is based on the drugs originated from plants, animal, and minerals. There is a need to maintain their purity, quality and safety by subjecting the finished products to rigorous scientific testing and to lay down pharmacopoeial standards for both Single and Compound drugs. Standardization of drugs is necessary especially in case of compound formulations to maintain the quality control and to identify the adulterated drugs. So Unani formulation namely *Habbe Mubarak* (HM) was subjected to standardization which is indicated in *Hummae Ajamiya* (malarial fever) and as *Dafe Tap* (antipyretic). The present study was designed to establish the physicochemical standards which can be used for future reference. The physicochemical standardization of HM includes organoleptic characters, weight variation of pill, uniformity in diameter, hardness test, friability test, pH, moisture content, loss of weight on drying, ash values, water and alcohol soluble matter, extractive values, disintegration time, thin layer chromatography (TLC) and total alkaloidal estimation. The findings of physicochemical study of the lab samples of HM may act as reference for quality control.

Keywords: Drug Standardization, Thin Layer Chromatography, *Myrica esculenta, Caesalpinia bonducella*

Introduction

In India, the herbal drug market is about $ one billion and the export of plant based crude drugs is around $ 80 million. But the most important challenges faced by these formulations arise because of their lack of complete standardization. Unani medicines are usually consists of plant origin drugs which are prone to contamination, deterioration and variation in composition. Therefore, quality control of herbal medicines offers a host of problems. To solve this problem, first and foremost task is the selection of the right kind of plant material which is therapeutically efficacious (Panchal *et al.,* 2011).

Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine. Hence standardization is a tool in the quality control process (Kunle *et al.,* 2012).

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This can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization. Therefore, the study was carried out to evaluate for various physicochemical parameters of the *Habbe Mubarak* (Anonymous, 2006) like ash value, moisture content, pH of 1% and 10% solutions, disintegration time, pill friability, hardness etc. Physicochemical standardization of Unani formulations is essential for the authenticity of the test compounds which provides standard modes for the preparation of the formulations and also describes salient features of identification of the product.

Methodology

To established Physicochemical standards of finished product of Habbe Mubarak (HM) it was prepared according to the formula described in National Formulary of Unani Medicine (NFUM) (Anonymous*,* 2006) as per WHO guidelines.

Ingredients

1.	Kaifal	*(Myrica esculenta*)	1 part
2.	Maghze Karanjwa	*(Caeslpinia bonducella*)	2 part

Procurement of Raw Drugs

All the ingredients of HM were procured from authorised raw drug dealer in Bangalore and identified by the Unani faculty members supervising the study. Voucher specimens (no. 20/IS/Res./2014) were deposited in the drug museum of NIUM.

Physicochemical Studies

The Physicochemical studies were carried out which included (i) Organoleptic characters of the huboob such as appearance, colour, smell, texture, taste; (ii) Weight variation of pill; (iii) Uniformity in diameter; (iv) Hardness test; (v) Friability test; (vi) pH; (vii) Moisture content; (viii) Loss of weight on drying; (ix) Ash values; (x) Water and alcohol soluble matter; (xi) Extractive values; (xii) Disintegration time; (xiii) Thin layer chromatography (TLC); (xiv) Total alkaloidal estimation.

Organoleptic Properties

Organoleptic evaluation refers to the evaluation of the formulation by colour, odour, taste and texture.The organoleptic characters of the pills were evaluated based on the method of Pandey *et al.* (2012) and Lachman *et al.* (2013).

Weight Variation

Twenty pills were selected randomly and their average weight was determined and pills were also weighed singly. The deviation from the average weight in each case was calculated and expressed as percentage. The pills meet the test, if not more than two pills are outside the limit of 5% (Anonymous*,* 2006; Lachman *et al.,* 2013).

Uniformity of Diameter

Uniformity of diameter was performed by picking three pills randomly and the diameter was measured individually by Vernier calliper and expressed in mm (Dandagi *et al.,* 2006).

Hardness Test

Three pills were taken and they were individually tested for the hardness by the Monsanto hardness tester in terms of kg/cm (Lachman *et al.,* 2013; William & Wilkins, 2011).

Friability Test

Friability of the pills was determined using Friability test apparatus (Roche's Friabilator) in which combined effect of abrasions and shock in a plastic chamber revolving at 25 rpm and dropping the pill at a height of 6 inches in each revolution. Pre weighed sample of pills was placed in the friabilator and after 100 revolutions collected and de-dusted using a soft muslin cloth and reweighed. The friability (¦) was calculated by the formula

$$\int = \left(1 - \frac{W}{W_0}\right) \text{x } 100$$

Where, W is the weight of the pills before the test and W_o is the weight of the pills after the test (Lachman *et al.,* 2013; Vijaya and Mishra, 2006).

Determination of pH

pH value of 1% and 10% solution: An accurately weighed 1 gm and 10 gm of powder drug was dissolved in accurately measured 100 ml of water separately, filtered and pH measured with a pH meter for 1 % and 10% respectively (Anonymous*,* 2006).

Moisture Content

The moisture content of the drug was determined by Toluene Distillation method. 10gm of powdered drug was taken in a flask of the apparatus and 75 ml of distilled

toluene added to it. Distillation was carried out for five hours. The volume of water collected in receiver tube was noted and the percentage of moisture calculated with reference to the weight of the air-dried drug taken (Jenkins *et al.,* 2008; Afaq *et al.,* 1994).

Loss of Weight on Drying at 105°C

Two gram of drug was taken, spread uniformly and thinly in a shallow petri dish. It was heated at a regulated temperature of 105ºC, cooled in a desiccator and weighed. The process was repeated many times till two consecutive weights were constant. The percent loss in weight was calculated with respect to initial weight (Anonymous, 2006; Afaq *et al.,* 1994).

Ash Values

Total Ash: Two gm of dried powdered drug was incinerated in a silica crucible at a temperature not exceeding 450ºC until free from carbon, cooled and weighed and the percentage was calculated with reference to dried drug.

Acid Insoluble Ash: Total ash was boiled with 25ml of dilute hydrochloric acid for 5 minutes. The insoluble matter was collected on an ash less filter paper washed with hot water and ignited at a temperature not exceeding 450ºC and weighed after cooling. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water Soluble Ash: Total ash was boiled with 25 ml of distilled water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited. The weight of insoluble ash was subtracted from the weight of the total ash, giving the weight of the water soluble ash. The percentage of water soluble ash was calculated with reference to air dried drug (Afaq *et al.,* 1994; Anonymous, 2006; Anonymous, 2011).

Determination of Water and Alcohol Soluble Matter

Accurately weighed 4 gm of drug was placed in a glass stoppered conical flask. Macerated with 100 ml of water for 6 hours shaking frequently, and then allowed standing for 18 hours, then shaked well and filtered rapidly through dry filter. 25 ml of the filtrate was transferred to a previously weighed and tarred flat-bottomed dish and evaporated to dryness on a water bath, then dried at 105ºC for 6 hours, cooled and weighed without delay. The percentage of water soluble matter was calculated with reference to the amount of drug taken. The alcohol soluble matter was determined as above by using alcohol in place of water (Anonymous, 2011; Anonymous, 2006).

Determination of Extractive values

Successive Extractive Value: The extractive values of pills in different solvents viz. Petroleum ether, alcohol and water were carried out by percolation in soxhlet apparatus. Powdered pills were taken and subjected to successive extraction with each solvent. The heat was applied for six hours on a heating mantle for each solvent. The extracts were filtered using filter paper and after evaporation of the solvents on water bath, the extractive values were determined with reference to the weight of drug (% w/w) (Anonymous, 2006).

Non-Successive Extractive Value: The extractive values of pills in different solvents viz. alcohol and water were carried out separately by percolation in soxhlet apparatus. The heat was applied for six hours on a heating mantle for alcohol and water. Powdered pills were taken and subjected to separate extraction with each solvent (% w/w). The extracts were filtered using filter paper and after evaporation of the solvents on water bath, the extractive values were determined with reference to the weight of drug (Anonymous, 2006).

Disintegration Time

The disintegration time was measured by Disintegration-testing apparatus using DDW as a medium at 37°C. Each of six pills was placed separately in the six cylinders of the two basket rack assemblies of the disintegration apparatus (Lachman *et al.,* 2013; William & Wilkins, 2011).

Alkaloidal Estimation

Five gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop by drop to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Sutharsingh *et al.,* 2011).

Thin layer Chromatography

Thin layer chromatography was carried out on T.L.C. pre coated aluminium plates, silica gel 60 F 254 (layer thickness 0.25 mm) for alcoholic extract of Habbe Mubarak in benzene: ethyl acetate (3: 1) as mobile phase and for spot detection iodine vapour was used. The R_f values of the spots were calculated by the following formula (Afaq *et al.,* 1994).

$$R_f \text{ value} = \frac{\text{Distance travelled by the spot}}{\text{Distance travelled by the solvent}}$$

Results and Discussion

Data of physicochemical evaluation of Habbe Mubarak is based on three readings for each parameter. The organoleptic characteristics i.e. appearance, colour, smell and taste of Habbe Mubarak were found to be spherical round like a pill, brown, non specific and bitter respectively which is the basis for identifying the drug (Table 1, Figure 1).

Table 1: Organoleptic Description of Habbe Mubarak

Appearance	Pill
Colour	Brown
Smell	Non specific
Texture	Hard
Taste	Bitter



Figure 1: Sample of Habbe Mubarak

Weight Variation of Pill: Test is helpful to ensure that a pill contains the proper amount of drug. The % of weight variation of the lab samples was within the prescribed limits of ± 5%. The mean value of randomly selected 20 pills was found to be 504.05± 1.87 mg. (Table 2).

The uniformity of diameter of the circular pills was also measured. The mean value of the diameter of HM was found to be 9.43 ± 0.03 mm. (Table 3).

Table 2: Weight variation of Habbe Mubarak

Sl.No.	Weight of individual Habb (mg)	Absolute difference in weight from mean (mg)	Weight variation (%)
1.	504	0.05	0.01
2.	489	15.05	2.98
3.	496	8.05	1.59
4.	510	5.95	1.18
5.	499	5.05	1.00
6.	497	7.05	1.39
7.	499	5.05	1.00
8.	493	11.05	2.19
9.	509	4.95	0.98
10.	494	10.05	1.99
11.	517	12.95	2.57
12.	503	1.05	0.21
13.	501	3.05	0.61
14.	505	0.95	0.19
15.	510	5.95	1.18
16.	508	3.95	0.78
17.	520	15.95	3.16
18.	504	0.05	0.01
19.	517	12.95	2.57
20.	506	1.95	0.39
Mean ± SEM	504.05 ± 1.87		

Hardness test is done to determine the force required to break the sample along its diameter. The mean value of the hardness of HM was found to be 4.4 ± 0.12 kg/cm (Table 3).

Friability test is done to determine the possible reduction in the weight of the solid dosage forms as a result of the mechanical erosion during handling and transportation. The mean percentage of friability of HM was found to be 0.19 ± 0.01 % (Table 3).

pH value of the drug is also an important parameter because weak acids would be better absorbed from the stomach than from the upper intestine. pH of HM was found to be slightly acidic for the drug and the value were found to be 6.30 ± 0.01 and 5.92 ± 0.01 in 1% and 10% aqueous solution respectively (Table 4).

Moisture content for detecting the quality of the drugs; excessive moisture content affects the quality of the drug and also its efficacy and more moisture becomes ideal medium for the growth of the bacteria and fungi which spoil the quality of the drug. The percentage of moisture content in HM was found to be 5.33 ± 0.33 (Table 4).

Table 3: Diameter, Hardness and Friability of Habbe Mubarak

Sl.No.	Diameter of Pill (mm)	Hardness (kg/cm)	Friability (%)
1.	9.5	4.6	0.20
2.	9.4	4.4	0.20
3.	9.4	4.2	0.17
Mean ± SEM	9.43 ± 0.03	4.4 ± 0.12	0.19 ± 0.01

Table 4: pH Values, Moisture content by Toluene Distillation Method and Loss of Weight on drying of Habbe Mubarak

Sl.No.	pH Values		Moisture Content (%)	Loss of weight on drying (%)
	1% Solution	10% Solution		
1.	6.32	5.92	5	5.15
2.	6.28	5.94	5	5.76
3.	4.30	5.90	6	6.14
Mean±SEM	6.30 ± 0.01	5.92 ± 0.01	5.33 ± 0.33	5.68 ± 0.29

Loss of weight on drying is done to determine the amount of water, volatile matter or mass in the sample. The percentage loss of weight on drying was found to be 5.68 ± 0.29 (Table 4).

Ash value of the drug is done for the detection of impurities and adulteration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it but it may also gives information related inorganic matter added for the purpose of adulteration of the drug. The mean percentage values of the total ash, acid insoluble ash and water soluble ash were found to be 11.95 ± 0.03 %, 0.43 ± 0.04 % and 3.21 ± 0.29 % respectively (Table 5).

Water and Alcohol soluble matter: The amount of extract that a drug yields to a given solvent is often an approximate measure and act as an index for some drugs. The mean percentages of alcohol and water soluble matter were found to be 12.93 ± 0.13 and 9.07 ± 0.08 respectively (Table 6).

Extractive values helps in the determination of the adulteration and is an index of the purity of the drug. The mean percentages of the non-successive extractive values were found to be 24.81 ± 1.28 and 14.88 ± 0.11 with water and alcohol, respectively and successive extractive values were found to be 11.77 ± 0.03, 5.62 ± 0.06 and 16.28 ± 0.03 in petroleum ether, alcohol and water, respectively (Table 7).

Disintegration test is done to determine whether tablets, capsules and pills disintegrate within the prescribed time and breaks down into smaller particles.

Table 5: Ash Value of Habbe Mubarak

Sl.No.	Total ash (%)	Acid insoluble ash (%)	Water soluble ash (%)
1.	11.98	0.49	3.78
2.	11.99	0.44	2.89
3.	11.89	0.34	2.95
Mean ± SEM	11.95 ± 0.03	0.43 ± 0.04	3.21 ± 0.29

Table 6: Alcohol and Water soluble matter of Habbe Mubarak

Sl.No.	Alcohol soluble matter (%)	Water soluble matter (%)
1.	13.15	9.23
2.	12.70	9.03
3.	12.93	8.95

The mean value of disintegration time in aqueous medium was found to be 29.33 ± 0.88 minutes (Table 8).

Total Alkaloids: As a medicinal agent, alkaloids are characterized by their high potency. The mean value of total alkaloidal estimation of HM was found to be 0.15 ± 0.02 % (Table 8).

Thin layer chromatography is an important parameter used for detecting the adulteration for analysing the quality and purity of the drugs. Six spots were found on TLC silica plate with the alcoholic extract of HM. The R_f values were found to be 0.014, 0.271, 0.518, 0.612, 0.776, and 0.906 (Table 9, Figure 2).

Table 7: Non-Successive and Successive Extractive Values of Habbe Mubarak

Sl.No.	Non-Successive Extractive Value		Successive Extractive Values		
	Water (%)	Alcohol (%)	Petroleum ether (%)	Alcohol (%)	Water (%)
1.	23.91	15.07	11.75	5.63	16.29
2.	27.34	14.89	11.82	5.51	16.22
3.	23.18	14.68	11.73	5.72	16.34
Mean ± SEM	24.81 ± 1.28	14.88 ± 0.11	11.77 ± 0.03	5.62 ± 0.06	16.28 ± 0.03

Table 8: Disintegration time and Total Alkaloidal Estimation of Habbe Mubarak

Sl.No.	Disintegration time in Aqueous medium (min)	Total Alkaloidal Content (%)
1.	29	0.18
2.	28	0.12
3.	31	0.16
Mean ± SEM	29.33 ± 0.88	0.15 ± 0.02

Table 9: TLC of Habbe Mubarak

Extract	Solvent	Treatment	No. of Spots	R_f Value	Colour
Ethanol	Benzene: Ethyl acetate (3: 1)	Iodine Vapour	6	0.014, 0.271, 0.518, 0.612, 0.776 and 0.906	Yellow



Figure 2: TLC of Habbe Mubarak

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Ingredient Identification in Unani formulation Aksir-e-Riyah - A Leading Step Towards Quality Assurance

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Abstract

Quality Assurance is the sum of the productive process/ steps performed with the objective of ensuring that products will be of quality, required as per WHO, GMP norms. Ingredient identification is a leading step to ensure the quality of herbal drugs by controlling all aspects of manufacturing process. In view of this present communication reports ingredient identification in Aksir-e-Riyah, a Unani compound formulation, which is considered as Kasir-e-Riyah (carminative) in Unani system of medicine. The drug is recommended by Unani physicians in cases of Qulanj (colic), Nafakh-e-Shikan (flatulence in the stomach) and Waj-ul-Kulya (nephralgia). All the ingredients that are required in the preparation are examined separately (both macroscopically as well as microscopically) followed by the microscopic examination of the formulation as a whole. This provides a set of diagnostic histological characters that serve a leading step in preparing a quality drug with maximum therapeutic potential and hence beneficial for the mankind.

Keywords: Ingredient identification, Quality assurance, Unani formulation.

Introduction

The prime objective of anyone, working in a herbal industry, is to ensure that products are constantly manufactured to quality, appropriate to their intended use and at the lowest possible cost. The attitude of general public towards quality is vastly different from what it was few years ago. There seems to be feeling that all marketed items are completely safe and are of good quality. Virtually every country in the world is conscious for herbal drugs, that is why quality is the main criterion of product license application.

Early attempt to control the quality of products were by the end product testing i.e. quality control approach. The pharmaceutical industry lead the change from quality control to quality assurance approach. Quality assurance is the sum of the production process/ steps performed with the object of ensuring that products will be of quality, required as per WHO, GMP norms. Ingredient identification is a leading step to ensure the quality of a herbal drug by controlling all aspects of manufacturing process. In view of this present communication reports ingredient identification in Aksir-e-Riyah, a Unani compound formulation,which is considered as Kasir-e-Riyah (carminative) in Unani system of medicine and is recommended by the Unani physicians in case of Qulanj (colic), Nafakh-e-Shikan (flatulence in the stomach) and Waj-ul-Kulya (nephralgia) (Kirtikar and Basu, 1988; Nadkarni,

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1986). All the ingredients that are required in the preparation are examined separately (both macroscopically as well as microscopically) followed by the microscopic examination of the formulation as a whole (Johansen, 1940; Trease and Evans, 1983). This will provide a set of diagnostic histological characters that serves a leading step in preparing a quality drug with maximum therapeutic potential and hence beneficial for the mankind.

Methodology

All the ingredients were procured from the local raw drug dealers, New Delhi. Each ingredient was authenticated (by examining both macroscopically and microscopically) and powdered separately. Aksir-e-Riyah was prepared as per formulation composition (Anonymous, 2007; Kirtikar and Basu, 1988; Nadkarni, 1986).

Formulation Composition:

S.No.	Ingredients	Scientific Name	Part used	Quantity
1.	Hilteet khalis	*Ferula foetida* Regel	Oleo-resin	8 kg.
2.	Kafoor khalis	*Cinnamomum camphora* Nees & Bem	Crystal	8 kg.
3.	Zanjabeel	*Zingiber officinale* Rosc	Rhizome	16 kg.
4.	Ajwayin khursani	*Hyoscyamus niger* Linn.	Seed	16 kg.
5.	Filfil siyah	*Piper nigrum* Linn.	Berries	16 kg.
6.	Gul-e-Aak	*Calotropis procera* (Ait.) R. Br.	Flower	16 kg.
7.	Zarambad	*Hedychium spicatum* Ham ex. Smith	Rhizome	16 kg.
8.	Soda khurdani	Sodium bicarbonate	Powder	24 kg.
9.	Namak siyah	Black salt	Crystal	16 kg.
10.	Namak-e-Turb	*Raphanus sativus* Linn.	Powder	16 kg.
11.	Naushadar	Ammonium chloride	Crystal	16 kg.

Observations

Ingredients

1. Hilteet Khalis

   Part used: Oleo resin

   Macroscopy: rounded, flattened or masses of agglutinated tears, grayish whit to dull yellow, approx. 12 – 25 mm. in diameter, opaque, odour – characteristic, strong; taste -bitter and acrid.

2. Zanjabeel *(Zingiber officinale* Rosc.)

   Part used: Rhizome

   Macroscopy: Rhizome irregularly branched (sympodial), laterally compressed, different sizes, externally pale yellowish-buff, longitudinally striate, ends of branches with depressed stem scars, fracture short, mealy, uneven with projecting fibres, odour agreeably aromatic with characteristic pungent taste.

   Microscopy: A cross section of rhizome shows:

   Phellem or outer cork few layered, dark brown, irregular parenchyma cells.

   Phellogen or inner cork few layered, colourless parenchyma cells, radially arranged in regular rows.

   Phelloderm or cortex several layered, thin walled, round- polygonal, parenchyma cells with intercellular spaces containing abundant starch grains which are mostly simple, fairly large, flattened, oblong or sub-rectangular to oval or sac shaped with terminal beak like projection in which eccentric hilum is situated. Numerous oleo- resin cells and vascular bundles present.

   Endodermis single layered with radial walls thickened, starch grains absent.

   Stele broad central zone, thin walled, round- polygonal, parenchyma cells with intercellular spaces (same as cortex) just inside the endodermis i.e. to the periphery of the ground tissue a ring or narrow zone of vascular bundle present. Scattered irregularly throughout the remainder of the stele are larger, closed, collateral, fibro-vascular bundles.

3. Ajwayin khursani *(Hyosyamus niger* Linn.)

   Part used: Seed

   Macroscopy: Dark grey seeds, approx. 1mm, reniform or sub-quadrate; odour – pleasantly aromatic; taste – pungent, bitter and mucilaginous.

Microscopy: T.S. of seed shows that testa consists of an outer layer of osteosclereids covered by thick cuticle; inner layer consists of crushed parenchyma cells; endosperm thin walled parenchymatous containing aleurone grains and oil globules.

4. Filfil siyah *(Piper nigrum* Linn.)

Part used: Berries

Macroscopy: Fruits globular, hard, dark brown to black, 3- 5mm. in diameter with a characteristic coat of deep set wrinkles; odour aromatic, taste pungent.

Microscopy: T.S. of fruit shows:

Epicarp: Single layered epidermis covered by cuticle; epidermal cells polygonal (tabular) containing dark brown- blackish content followed by 2- 3 layers of thin walled parenchyma cells intermingled with thick walled isodiametric to radially elongated lignified stone cells.

Mesocarp: Broad zone of tangentially elongated parenchyma cells having larger secretion sacs with sub-erised walls and oil or resin contents. Cells in the inner mesocarpic region are compressed having few fibro vascular bundles.

Endocarp: Single row of beaker shaped stone cells (cells whose radial and inner walls are more strongly lignified than the outer ones).

Testa: Single layer of yellow coloured cells.

Perisperm: Broad zone of thin walled, radially elongated parenchyma cells filled with abundant starch grains, aleurone grains, oleoresin cells containing oil globules and masses of resin.

5. Gul-e-Aak *(Calotropis procera* (Ait.) Bru.)

Part used: Flower

Macroscopy: Pentamerous flower, calyx divided to the base, sepal ovate, acute, glabrous, corolla whitish outside and violet inside, lobes of corona compressed equaling the staminal column, stigma fused with androceium forming gynostagium, all pollen grains of each lobe aggregate together to form pollinium with a stalk called caudicle and sticky base called disc of corpusculum.

Microscopy: T.S. of pedicle shows single layered epidermis covered by cuticle, trichomes present; cortex several layered, parenchymatous having abundant branchy sclereids of varying size, vascular bundle present at the centre.

Cross secion of sepal and petal shows an upper and lower epidermis having numerous hairs and several layers of thin walled parenchyma cells in between. Cells of petals contain violet pigments.

Carpel shows bicarpellary ovary containing numerous ovules.

6. Zarambad *(Hedychium spicatum* Ham. ex Smith.)

Part used: Rhizome

Macroscopy: Rhizome dry, dark brown, various sizes showing rudiments of root-lets, surface marked with numerous scars and circular rings, taste bitter, odour camphoraceous.

Microscopy: T.S. of rhizome shows: -

Phellem or outer cork few layered, dark brown, irregular parenchyma cells.

Phellogen or inner cork few layered, light brown parenchyma cells, radially arranged in regular rows.

Phelloderm or cortex several layered, thin walled, round- polygonal, parenchyma cells with intercellular spaces containing abundant starch grains which are mostly simple, oval – oblong. Numerous oleo-resin cells filled with greenish yellow oil globules.

Endodermis consisting of single layered, parenchymatous.

Vascular bundles closed, collateral, scattered irregularly throughout the ground tissue.

Test Sample (Formulation):

Microscopic examination of Aksir-e-Riyah shows following components of diagnostic characteristics: -

Epidermal cells: Fragments of epidermal cells in surface v iewshowing stomata and non-glandular, unicellular trichomes.

Fibers: Pieces of fibres of different size, present either single or associated with vessels.

Palisade cells: Fragment of testa showing columnar palisade cells.

Parenchyma cells: Numerous, different size and shape, present either single or in groups, mostly filled with starch grains, some contains oil globules.

Starch grains: Abundant, present either scattered or within the parenchyma cells, various size and shape, some are simple, fairly large, flattened, oblong with a small pointed hilum situated at the narrower end, some are irregularly ovoid – ellipsoidal, showing tuberosites,

Stone cells: Present either single or in groups, nearly isodiametric.

Trichomes: Unicellular, non glandular, unseptate.

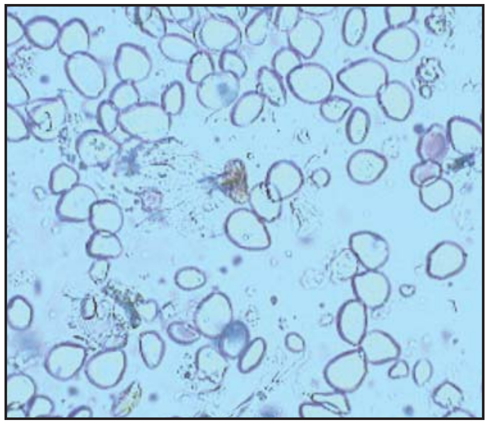


Fig. 1 x40 Starch grains of Zanjabeel

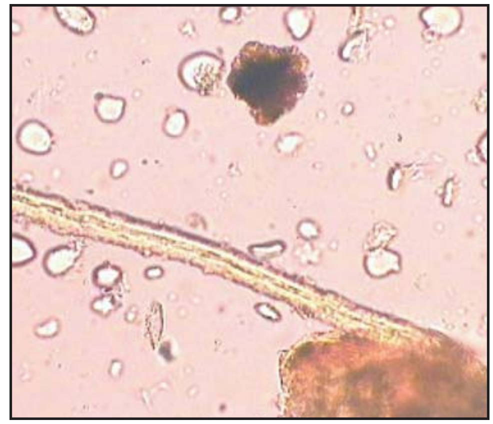


Fig. 2 x40 Fibre of Zanjabeel



Fig. 3 x40 Piece of vessel showing reticulate thickening of Zanjabeel



Fig. 4 x100 Vascular elements of Zanjabeel

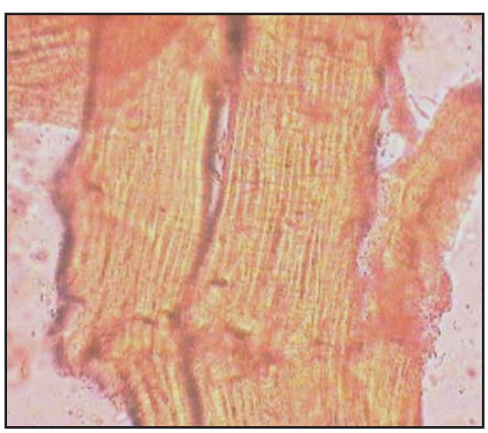


Fig. 5 x40 Fragment of testa of Ajwayin khurasani

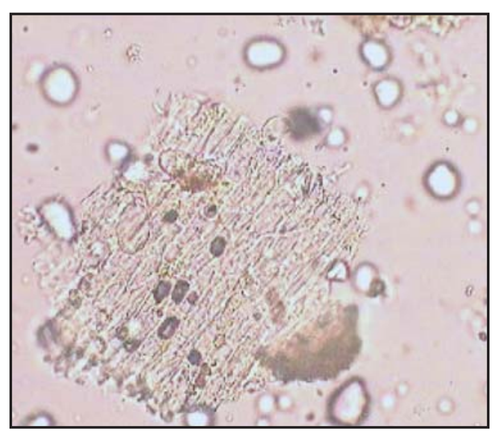


Fig. 6 x40 Parenchyma cells of Ajwayin khurasani

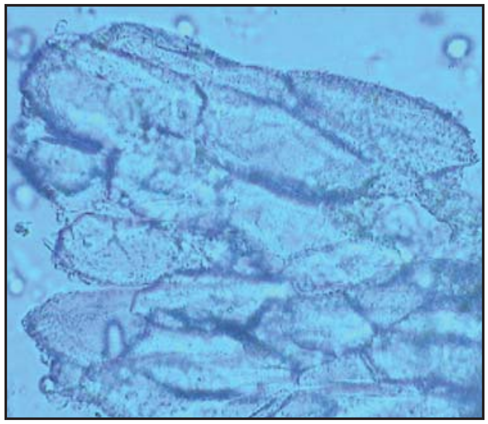


Fig. 7 x40 Parenchyma cells filled with starch grains of Filfil Siyah



Fig. 8 x40 Sclereid of Filfil Siyah

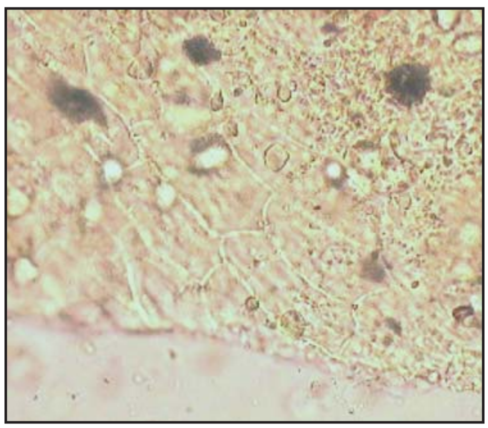


Fig. 9 x40 Epidermal cells of Gul-e-Aak



Fig.10 x40 Trichome of Gul-e-Aak



Fig.11 x40 Epidermal cell showing stomata in Gul-e-Aak

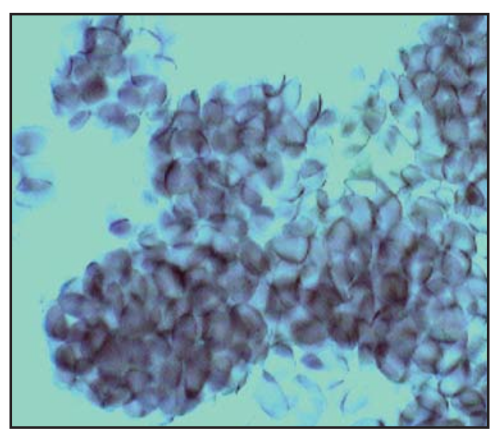


Fig. 12 x40 Parenchyma cells filled with starch grains in Zarambad

Results and Conclusion

Aksir-e-Riyah is yellowish brown powder with salty taste and pungent odour. On the basis of above mentioned histological characters, presence of following ingredients was established in Aksir-e-Riyah: -

- Abundant starch granules which are simple, fairly large, flattened, oblong with a small pointed hilum situated at the narrower end, pieces of fibreseiher single or found associated with vessels, pieces of vessels which are reticulately thickened (Zanjabeel) - fig. 1-4.
- Fragment of testa showing columnar palisade cells, thin walled parenchyma cells containing oil globules (Ajwayin khursani) fig. 5,6.
- isodiametric stone cells and parenchyma cells filled with abundant minute starch grains
- (Filfil siyh) fig. 7,8.
- fragment of epidermal cells showing stomata and non glandular, unicellular trichomes
- (Gul-e-Aak) fig. 9-11
- parenchyma cells filled with numerous starch granules which are single, irregularly ovoid-ellipsoidal, occasionally showing tuberosites (Zarambad) fig. 12.

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Anticandidal Activity of Ornamental *Punica granatum* Linn. Flowers

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Abstract

Punica granatum Linn. is a shrub belonging to the family Punicaceae. It is often a cross pollinated food crop and has large forms of cultivars in various regions of the world. Double flower – *Punica granatum* is an ornamental type where the flowers have numerous petals and look like a rose flower and have a significant ornamental value. In Unani system of medicine, these flowers are called as "Gulnar" and are used as one of the ingredient in many formulations. Though the flower has significant therapeutic use since antiquity, many fundamental aspects including pharmacological and microbiological activities are lacking. Hence, in the present study, the anticandidal potency of the flowers of *Punica granatum* was studied against the clinical strains of *Candida albicans* collected from various laboratories and hospitals responsible for causing oral thrush or candidiasis in human. The alcoholic extract of the flowers found to have good anticandidal effect and had a significant control against most of the strains tested. The MIC dosage level was also determined.

Keywords: Anticandidal activity, *Punica granatum* L., Candiasis

Introduction

The use of natural products with therapeutic properties is as ancient as human civilization. For a long time, mineral, plant and animal products were the main sources of drugs (De Pasquale, 1984). In recent years, throughout the world, there has been a growing interest in alternative therapies and the therapeutic use of natural products especially those derived from plants (Goldfrank *et al*., 1982; Vulto and Smet, 1988). In India, traditional medicine is mainly based on three major systems of medicines namely, Ayurveda, Siddha and Unani. In almost all the system, the medicinal plant plays a major role and constitutes the backbone. Among various plants of commercial value, pomegrante (*Punica granatum*) is known worldwide for its delicious taste and health promoting properties. The plant *Punica granatum* is a member of the Punicaceae that has a busy appearance, tends to develop multiple trunks, and grows upto a height of 12 to 16 feet (Sharrif and Kashani, 2012).

Varietal wealth of *Punica granatum*

In different pomegranate growing areas of the world a good number of varieties have been identified by growers and breeders and cultivated either as commercial crop for the production of pomegranates or as ornamental crop (Jaliop, 2010).

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Double flowers of *Punica granatum*

Double flower bearing *Punica granatum* is an ornamental type and finds a significant use in horticulture for its beautiful flowers. The plant is considered to be native of Iran, Arabia, Afghanistan and Baluchistan and cultivated all over India. It grows in the hill slopes of Jammu & Kashmir and Himachal Pradesh between 900m and 1800m. The plant mostly flowers in April - June, but may occur even at other seasons also (Dina Nath Tiwari *et al.,* 2001). Double flower as the name implies are large attractive and look like rose flowers, wherein numerous stamens are modified into petals. Due to these modifications, some of these cultivars do not set fruits (abortive). As per the literature survey the commercial samples of the Unani drug used under the name Gulnar have been identified as the flowers of the horticulture form of *Punica granatum* (which does not produce fruits) (Tiwari *et al., 2001).*

Medicinal uses of *Punica granatum* double flowers

Gulnar - the abortive double flowers of *Punica granatum, in* Unani system of medicine is prevalently used in various forms like decoction, powder, syrup, infusion, nasal drop, gargle and pessery for treating various ailments (Nazamuddin *et al.,* 2013). These flowers have different pharmacological activities and being used as astringent, haemostatic, antihelmintic, stomachic, desiccant and cicatrizant. It is also used as one of the ingredients in many formulations prescribed by the Unani physicians for treating various ailments.

Oral thrush or Candidiasis

Oral thrush or candidiasis is the fungal infection caused by the yeast *Candida albicans*. The organism is usually present as commensals in all human skin and mucous membranes. Infection occurs when there is overgrowth of the organisms with signs and symptoms like; (i) creamy white lesions with a cottage cheese-like appearance on tongue, inner cheeks, on the roof of mouth, gums, tonsils; (ii) redness or soreness that may be severe to cause difficulty while eating or swallowing; (iii) slight bleeding if the lesions are rubbed or scraped; (iv) cracking and redness at the corners of mouth; (v) loss of taste etc. The infection occurs mainly to those who have either diabetes, glandular (endocrine) disorders, genetic disorders such as down syndrome, leukemia, lymphoma, immunodeficiency such as HIV/AIDS or those who have underwent a course of oral antibiotics especially inhaled steroids for certain lung condition, chemotherapy etc (Mariappan *et al.,* 2011).

With this view, the present study was designed to determine the anticandidal activity of ethanol extract of ornamental flowers of *P.granatum* against the strains of *Candida albicans* responsible for oral thrush.

Materials and Methods

Collection of study material

Double flowers of *P. granatum* were collected from Herbal Garden of Regional Research Institute of Unani Medicine, Royapuram, Chennai, Tamil Nadu, during the month of April to June 2012 and were authenticated by the Botanist, Plant Anatomy Research Centre, Chennai, India (Figure-1).

Preparation of ethanol extract of flowers

Fresh abortive double flowers of ornamental type of *Punica granatum* were collected, shade dried at room temperature and coarsely powdered (Sieve No.10). Ten grams of the powder was extracted with ethanol using Soxhlet apparatus for 6 hrs. The extract was filtered using sterile Whatman filter paper No.2, dried using rotary vacuum filter at 40ºC.

Collection of microorganism

Swab samples (taken from the mouth along with the history of the patients) were obtained from various hospitals and laboratories, Chennai.

Confirmation for *Candida albicans* was performed in the Department of Microbiology, RRIUM, Chennai by performing the following tests (Myers and Koshis, 1982)

- Smear test
- Germ tube test
- Culture test

Five confirmed strains of *Candida albicans* coded as CAI, CAII, CAIII, CAIV, CAV and *Candida albicans* ATCC 10231 were stored in the SDA plates for further analysis of anticandidal study.

Anticandidal activity

The in-vitro anticandidal activity was performed using the cup plate method (Anonymous, 1996). The required number of Muller Hinton agar plates were prepared and swabbed with different isolates of log phase cultures of above mentioned *Candida albicans* cultures coded CAI, CAII, CAIII, CAIV, CAV and *Candida albicans* ATCC 10231. The plates were allowed to stand for few minutes. Required numbers of 6 mm diameter wells were made over the plates at an equidistant position. Wells were loaded with 70µl of the drug at the concentration of 100 mg/ml. Dimethylsulphoxide (DMSO) was used as the solvent. Separate

control disc was also included using the solvent. The ampicillin (10 mcg) dissolved in sterile DMSO was used as standard for comparison. All the plates were kept at 37ºC for 18-24 hrs. The zone of inhibition was measured using the calipers.

Minimum inhibitory concentration (MIC)

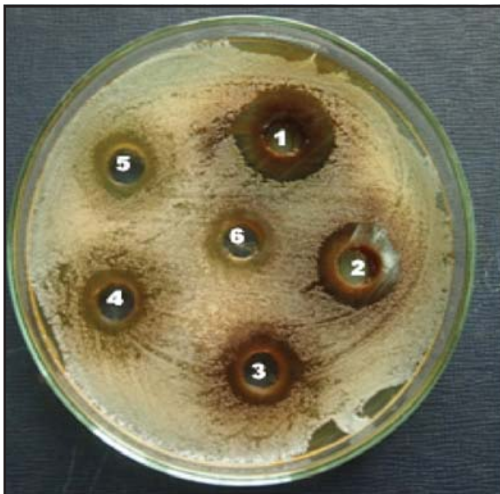
The MIC, the lowest concentration of the extract required to inhibit the microorganism was determined by the agar dilution method (Myers and Koshis, 1982). Different concentrations of alcohol flower extract ranging from 100 ìg/ìl, 50 ìg/ìl, 25 ìg/ìl, 12.5ìg/ìl, 6.25ìg/ìl and 3.125 ìg/ìl were used in the study. The lowest concentration of the drug (MIC) that completely inhibits the growth was determined after overnight incubation at 37°C for 18 to 24 hrs. The zone of inhibition was measured using the calipers (Table-1; Figure-2).

Table 1: MIC value

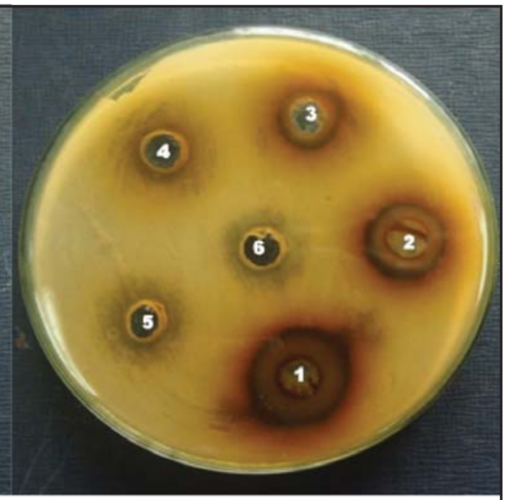
S.No.	Culture tested	MIC μg/μl	Std (Amp) mcg/ml
1.	*Candida albicans* CAI	3.125	Intermittently sensitive
2.	*Candida albicans* CAII	6.25	
3.	*Candida albicans* CAIII	6.25	
4.	*Candida albicans* CAIV	3.125	
5.	*Candida albicans* CAV	3.125	
6.	*Candida albicans* ATCC 10231	12.5	



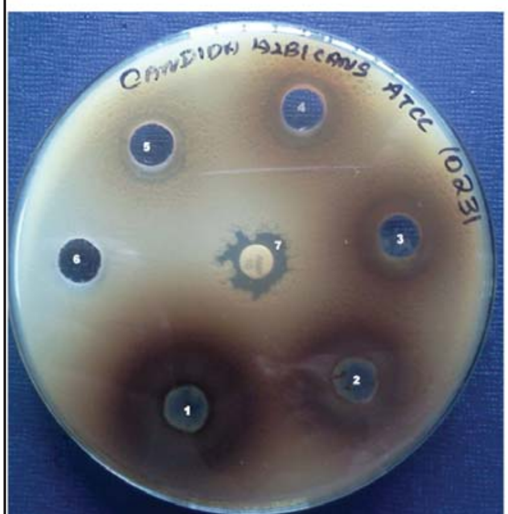
Figure 1: Double flower of *Punica grantum* Linn.



Candida albicans **- CA1**



Candida albicans **- CA3**



Candida albicans **- ATCC 10231**

Drug concentration

1. 100 μg/μl
2. 50 μg/μl
3. 25 μg/μl
4. 12.5 μg/μl
5. 6.25 μg/μl
6. 3.125 μg/μl
7. Ampicillin 10mcg.

Figure 2: Plates showing Anticandial activity

Results and Discussion

Oral hygiene and topical antifungal gels are usually adequate for the uncomplicated oral candidiasis. Modern drugs presently used in the treatment of *Candida albicans* are Gentian violet an aniline dye, but because of their staining of oral mucosa is replaced by other drugs like nystatin, Amphotericin B, Clotrimazole, Ketoconazole and Fluconazole which has its own potential side effects. In this situation, as per our research findings, the alcohol extract of the flowers of ornamental *Punica granatum* finds to possess good controlling capacity against the tested strains of *candida albicans*. Hence the study can be concluded as, the double flowers of

Punica granatum could be given as an alternative medicine either separately or in combination with modern medicine in the treatment of patients having oral thrush.

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